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(56) Related Art
Perelle S et al, (2004) "Detection by 5'-nuclease PCR of Shiga-toxin producing Escherichia coli O26, O55, O91, O103, O111, O113, O145 and O157:H7, associated with the world's most frequent clinical cases" Mol Cell Probes 18, 185–192
Genbank Accession No. AF461169, 1 July 2002
Genbank Accession No. EF441617, 21 December 2007

ABSTRACT

The disclosed invention is related to methods, compositions and kits for targeting nucleic acid derived from Shiga toxin-producing bacteria such as E. coli. Compositions include amplification oligomers and/or detection probe oligomers. Kits and methods comprise at least one pair of amplification oligomers.

DETECTION OF SHIGA TOXIN GENES IN BACTERIA

CROSS-REFERENCE TO RELATED APPLICATIONS

This present application is a non-provisional application and claims the benefit of 61/603,091, filed February 24, 2012, and 61/725,401, filed November 12, 2012, both of which are incorporated by reference in their entireties for all purposes. The present application is a divisional application of Australian Patent Application No. 2013205010 (PCT/US2013/027457), the content of which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to the detection of infectious agents, more specifically to the detection of Shiga toxin genes. Compositions, methods and kits are described for the detection of Shiga toxin genes by using *in vitro* nucleic acid amplification techniques.

BACKGROUND OF THE INVENTION

Escherichia coli (*E. coli*) is a gram-negative, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms. Most strains of *E. coli* are non-pathogenic and are part of the normal gut flora. However, some serotypes can cause life-threatening infections in humans. Pathogenic strains of *E. coli* can cause gastroenteritis, urinary tract infections, neonatal meningitis, haemolytic-uremic syndrome, peritonitis, mastitis, septicemia and pneumonia.

E. coli has the ability to transfer DNA to and from other bacteria. This ability has allowed some strains of *E. coli* to acquire the bacteriophage carrying the genes encoding Shiga toxin from *Shigella*. There are two main types of Shiga toxin produced by certain strains of *E. coli*, Shiga toxin type 1 and Shiga toxin type 2, which are carried on two different genes, *stx1* and *stx2*, respectively. Some strains of *E. coli* contain the *stx1* gene while other strains contain the *stx2* gene. There are also certain strains of *E. coli* that contain both the *stx1* and *stx2* genes. The most common strain of Shiga toxin-producing *E. coli* (STEC) in North America is 0157:H7, however, there are over one hundred strains of *E. coli* that can produce Shiga toxin. In addition to *E. coli*, *Citrobacter freundii*, *Aeromononas hydrophila*, *Aeromononas caviae*, and *Enterobacter cloacae* have also been reported to be able to produce Shiga toxin.

Shiga toxins bind to specific cell surfaces via glycolipid Gb3. Once bound to the cell, Shiga toxins enter the cell and shut down protein synthesis, which leads to cell death. In STEC, the Shiga toxin binds to the vascular endothelium of small blood vessels. The killing of vascular endothelium cells leads to the breakdown of the lining of the small blood vessels, which in turn results in haemorrhaging. Because STEC infections usually occur from ingesting contaminated food or water, the first symptom is generally bloody diarrhea. As the infection progresses, the toxin can spread to the kidneys, causing haemolytic uremic syndrome which affects the lungs, and nervous system. STEC infections occur in the United States at a rate of about 0.9 individuals per 100,000 for *E. coli* 0157 and at a rate of about 1 individual per 100,000 for strains other than 0157.

Routine methods for detecting Shiga toxin producing *E. coli* (STEC) involve analysis of stool cultures and/or enzyme immunoassays. Stool culture takes approximately 24 hours when grown on defined media. However, selective and differential media are only available for identifying 0157:H7. Because of the lack of media available, most labs do not have the capabilities to test for non-0157 STEC serotypes. Labs are required to send non-O157 isolates to their local or state labs for more complex testing such as pulse-field gel electrophoresis typing and virulence gene characterization. This is beyond the capabilities of many labs. Enzyme immunoassays (EIAs) detect Shiga toxins via anti-Shiga toxin capture antibodies absorbed to micro-wells. Diluted samples are added to the wells and incubated, washed and then anti-Shiga toxin antibodies are added. This is followed by more incubation and washing. Enzyme conjugated anti-IgG polyclonal antibody is added and incubated and if a Shiga toxin is present, a reactive antibody-enzyme complex is formed and a substrate solution is added to elicit a colored response in samples containing the Shiga toxin. A stop solution is then added and the results are interpreted. The EIAs may be used directly on stool specimens. However, these procedures have been found to be more sensitive if the stool specimen is subject to overnight broth enrichment. These EIAs can detect O157 and non-O157 STEC strains but with the need to incubate stool specimens overnight to maximize sensitivity, the tests take too long to generate answers. Thus, there is a need for an assay for the detection of STEC from a number of *E. coli* strains. There is a need in the art for an assay that rapidly detects STEC. There also is a need for an assay that specifically detects STEC. Additionally, there is a need for an assay that detects STEC with a high degree of sensitivity.

SUMMARY OF THE INVENTION

In a first aspect, the present invention provides a primer-probe set when used for identification of a stx1 gene, said primer-probe set comprising:

a pair of amplification oligomers, each member of said pair of amplification oligomers having a length of from about 15 to about 25 contiguous nucleotides, said pair of amplification oligomers comprising

(i) a first oligomer having at least 90% sequence identity to SEQ ID NO:30 and a second oligomer having at least 90% sequence identity to SEQ ID NO:31; and

an oligomer probe hybridizable to a stx1 gene region located between the regions of hybridization of said pair of amplification oligomers, wherein the probe comprises a detectable label.

In a second aspect, the present invention provides a kit when used for identification of a stx1 gene, said kit comprising the primer-probe set as recited in the first aspect.

In a third aspect, the present invention provides a kit when used for identification of a stx1 gene and a stx2 gene, said kit comprising the primer-probe set as recited in the first aspect and the primer-probe set for identification of a stx2 gene comprising:

(1) a pair of amplification oligomers, each member of said pair of amplification oligomers having a length of from about 15 to about 25 contiguous nucleotides, said pair of amplification oligomers comprising an oligomer pair selected from the group consisting of:

(i) a first oligomer having at least 90% sequence identity to SEQ ID NO:33 and a second oligomer having at least 90% sequence identity to SEQ ID NO:34;

(ii) a first oligomer having at least 90% sequence identity to SEQ ID NO:40 and a second oligomer having at least 90% sequence identity to SEQ ID NO:41;

(iii) a first oligomer having at least 90% sequence identity to SEQ ID NO:36 and a second oligomer having at least 90% sequence identity to SEQ ID NO:37; and

(iv) a first oligomer having at least 90% sequence identity to SEQ ID NO:47 and a second oligomer having at least 90% sequence identity to SEQ ID NO:48; and

(2) an oligomer probe hybridizable to a stx2 gene region.

In a fourth aspect, the present invention provides a primer-probe set when used for identification of at least one of a stx1 gene and a stx2 gene in a sample, said primer-probe set comprising:

a pair of stx1-specific amplification oligomers and a pair of stx2-specific amplification oligomers, each member of said stx1-specific and stx2-specific pairs of amplification oligomers having a length of from about 15 to about 25 contiguous nucleotides,

said pair of stx1-specific amplification oligomers comprising

(1-i) a first oligomer having at least 90% sequence identity to SEQ ID NO:30 and a second oligomer having at least 90% sequence identity to SEQ ID NO:31; and

said pair of stx2-specific amplification oligomers comprising an oligomer pair selected from the group consisting of

(2-i) a first oligomer having at least 90% sequence identity to SEQ ID NO:33 and a second oligomer having at least 90% sequence identity to SEQ ID NO:34;

(2-ii) a first oligomer having at least 90% sequence identity to SEQ ID NO:40 and a second oligomer having at least 90% sequence identity to SEQ ID NO:41;

(2-iii) a first oligomer having at least 90% sequence identity to SEQ ID NO:36 and a second oligomer having at least 90% sequence identity to SEQ ID NO:37; and

(2-iv) a first oligomer having at least 90% sequence identity to SEQ ID NO:47 and a second oligomer having at least 90% sequence identity to SEQ ID NO:48;

a stx1-specific detection probe comprising a detectable label, wherein the detection probe is hybridizable to a stx1 gene region located between the regions of hybridization of said pair of stx1-specific amplification oligomers; and

a stx2-specific detection probe hybridizable to a stx2 gene region located between the regions of hybridization of said pair of stx2-specific amplification oligomers.

This summary lists several embodiments of the presently disclosed subject matter, and in many cases lists variations and permutations of these embodiments. This summary is merely exemplary of the numerous and varied embodiments. Mention of one or more representative features of a given embodiment is likewise exemplary. Such an embodiment can typically exist with or without the feature(s) mentioned; likewise, those features can be applied to other embodiments of the presently disclosed subject matter, whether listed in this summary or not. To avoid excessive repetition, this summary does not list or suggest all possible combinations of such features.

The present invention relates to methods, compositions, kits and amplification products for identification of stx1 and stx2 genes.

One aspect of the invention relates to a method for identifying a stx1 gene in a sample. The method includes the step of contacting the sample with a pair of amplification oligomers. Each member of the pair of amplification oligomers has a length of from about 15 to about 25 contiguous nucleotides. The pair of amplification oligomers typically includes an oligomer pair selected from the group consisting of (i) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:30 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:31; (ii) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:1 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:2; (iii) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:8 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:9; (iv) a first oligomer having at least 90%, at least 95%, or 100% sequence identify to SEQ ID NO:12 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:13; and (v) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:19 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:20. The method includes the step of amplifying nucleic acid in the sample with the pair of amplification oligomers to obtain an amplification product. The method also includes the step of determining the sequence of the amplification product or detecting the amplification product using a detection probe.

In certain embodiments of the method for identifying a stx1 gene, the sample may contain bacterial nucleic acid originating from Escherichia coli, Citrobacter freundii, Aeromononas hydrophila, Aeromononas caviae, or Enterobacter cloacae. In certain embodiments of the method for identifying a stx1 gene, the bacterial nucleic acid originates from a strain of E. coli such as E. coli 0157:H7.

In certain embodiments of the method for identifying a stx1 gene, the amplification step is performed using the polymerase chain reaction. In certain embodiments, the polymerase chain reaction is a real-time polymerase chain reaction.

In certain embodiments of the method for identifying a stx1 gene, the detection probe is a capture probe or a fluorescence probe. The fluorescence probe can include a fluorescent dye compound and a non-fluorescent quenching dye compound.

In certain embodiments of the method for identifying a stx1 gene, the probe is an oligomer having a length of from about 15 to about 30, to about 35, or to about 40 contiguous oligomer residues. In certain embodiments of the method for identifying a stx1 gene, (i) for an amplification oligomer pair corresponding to SEQ ID NOS:30 and 31, the probe has at least 90%, at least 95% or 100% sequence identity to SEQ ID NO:32; (ii) for an amplification oligomer pair corresponding to SEQ ID NOS:1 and 2, the probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:5; (iii) for an amplification oligomer pair corresponding to SEQ ID NOS:8 and 9, the probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:11; (iv) for an amplification oligomer pair corresponding to SEQ ID NOS:12 and 13, the probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:14; or (v) for an amplification oligomer pair corresponding to SEQ ID NOS:19 and 20, the probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:22.

In certain embodiments of the method for identifying a stx1 gene, the step of determining the sequence of the amplification product is performed by a sequencing reaction, a microarray, electrophoresis, or mass spectrometry.

Another aspect of the invention relates to a primer pair for amplification of a stx1 gene. The primer pair includes a pair of amplification oligomers with each member of the pair typically having a length of from about 15 to about 25 contiguous nucleotides. In particular embodiments, the pair of amplification oligomers includes an oligomer pair selected from the group consisting of (i) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:30 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:31; (ii) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:1 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:2; (iii) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:8 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:9; (iv) a first oligomer having at least 90%, at least 95%, or 100% sequence identify to SEQ ID NO:12 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:13; and (v) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:19 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:20.

Another aspect of the invention relates to a primer-probe set for detection of a stx1 gene. The primer-probe set includes a pair of amplification oligomers with each member of the pair typically having a length of from about 15 to about 25 contiguous nucleotides. In particular embodiments, the primer probe set includes (1) a pair of amplification oligomers selected from the group consisting of (i) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:30 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:31; (ii) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:1 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:2; (iii) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:8 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:9; (iv) a first oligomer having at least 90%, at least 95%, or 100% sequence identify to SEQ ID NO:12 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:13; and (v) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:19 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:20; and (2) an oligomer probe which is hybridizable to a stx1 gene region located between the regions of hybridization of the pair of amplification oligomers. In certain embodiments, the probe has a length of from about 10 to about 30, to about 35, or to about 40 contiguous oligomer residues. In certain embodiments, (i) for an amplification oligomer pair corresponding to SEQ ID NOS:30 and 31, the probe has at least 90%, at least 95% or 100% sequence identity to SEQ ID NO:32; (ii) for an amplification oligomer pair corresponding to SEQ ID NOS:1 and 2, the probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:5; (iii) for an amplification oligomer pair corresponding to SEQ ID NOS:8 and 9, the probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:11; (iv) for an amplification oligomer pair corresponding to SEQ ID NOS:12 and 13, the probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:14; or (v) for an amplification oligomer pair corresponding to SEQ ID NOS:19 and 20, the probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:22. In certain embodiments, the primer-probe set further includes an internal control system for verifying reaction conditions. The system includes a control template polynucleotide, a pair of control amplification oligomers and a control probe.

Another aspect of the invention relates to a method for identifying a stx2 gene in a sample. The method includes the step of contacting the sample with a pair of amplification oligomers. Each member of the pair of amplification oligomers has a length of from about 15 to about 25 contiguous nucleotides. The pair of amplification oligomers typically includes an oligomer pair selected from the group consisting of (i) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:33 and a second oligomer having at least 90%, a least 95%, or 100% sequence identity to SEQ ID NO:34; (ii) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:40 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:41; (iii) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:36 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:37; and (iv) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:47 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:48. The method includes the step of amplifying nucleic acid in the sample with the pair of amplification oligomers to obtain an amplification product. The method also includes the step of determining the sequence of the amplification product or detecting the amplification product using a detection probe.

In certain embodiments of the method for identifying a stx2 gene, the sample may contain bacterial nucleic acid originating from E. coli, Citrobacter freundii, Aeromononas hydrophila, Aeromononas caviae, or Enterobacter cloacae. In certain embodiments of the method for identifying a stx2 gene, the nucleic acid originates from a strain of Escherichia coli such as E. coli 0157:H7.

In certain embodiments of the method for identifying a stx2 gene, the amplification step is performed using the polymerase chain reaction. In certain embodiments, the polymerase chain reaction is a real-time polymerase chain reaction.

In certain embodiments of the method for identifying a stx2 gene, the detection probe is a capture probe or a fluorescence probe. The fluorescence probe can include a fluorescent dye compound and a non-fluorescent quenching dye compound.

In certain embodiments of the method for identifying a stx2 gene, the probe is an oligomer having a length of from about 15 to about 30, to about 35, or to about 40 contiguous oligomer residues. In certain embodiments of the method for identifying a stx2 gene, for the amplification oligomer pair of (i), the probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:35; for the amplification oligomer pair of (ii), the probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:42; for the amplification oligomer pair of (iii), the probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:39, or (iv) for the amplification oligomer pair of (iv), the probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:49.

In certain embodiments of the method for identifying a stx2 gene, the step of determining the sequence of the amplification product is performed by a sequencing reaction, a microarray, electrophoresis, or mass spectrometry.

Another aspect of the invention relates to a primer pair for amplification of a stx2 gene. The primer pair includes a pair of amplification oligomers with each member of the pair having a length of from about 15 to about 25 contiguous nucleotides. The pair of amplification oligomers includes an oligomer pair selected from the group consisting of (i) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:33 and a second oligomer having at least 90%, a least 95%, or 100% sequence identity to SEQ ID NO:34; (ii) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:40 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:41; (iii) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:36 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:37; and (iv) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:47 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:48.

Another aspect of the invention relates to a primer-probe set for detection of a stx2 gene. The primer probe set includes a pair of amplification oligomers with each member of the pair typically having a length of from about 15 to about 25 contiguous nucleotides. In particular embodiments, the primer-probe set includes (1) a pair of amplification oligomers selected from the group consisting of (i) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:33 and a second oligomer having at least 90%, a least 95%, or 100% sequence identity to SEQ ID NO:34; (ii) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:40 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:41; (iii) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:36 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:37; and (iv) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:47 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:48; and (2) an oligomer probe which is hybridizable to a stx2 gene region located between the regions of hybridization of the pair of amplification oligomers. In certain embodiments, the probe has a length of from about 10 to about 30, to about 35, or to about 40 contiguous oligomer residues. In certain embodiments, for the amplification oligomer pair of (i), the probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:35; for the amplification oligomer pair of (ii), the probe has at least 90%, at least

95%, or 100% sequence identity to SEQ ID NO:42; for the amplification oligomer pair of (iii), the probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:39, or (iv) for the amplification oligomer pair of (iv), the probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:49. In certain embodiments, the primer-probe set further includes an internal control system for verifying reaction conditions. The system includes a control template polynucleotide, a pair of control amplification oligomers and a control probe.

Another aspect of the invention relates to a kit for amplification of a stx1 gene. The kit includes the primer pair for amplification of a stx1 gene as described above, optionally in combination with instructions for carrying out a polymerase chain reaction using the amplification oligomers.

Another aspect of the invention relates to a kit for detection of a stx1 gene. The kit includes at least one of the embodiments of the primer-probe sets for amplification of a stx1 gene as described above, optionally in combination with instructions for carrying out a polymerase chain reaction using the amplification oligomers.

Another aspect of the invention relates to a kit for amplification of a stx2 gene. The kit includes at the primer pair for amplification of a stx2 gene as described above, optionally in combination with instructions for carrying out a polymerase chain reaction using the amplification oligomers.

Another aspect of the invention relates to a kit for detection of a stx2 gene. The kit includes at least one of the embodiments of the primer-probe sets for amplification of a stx2 gene as described above, optionally in combination with instructions for carrying out a polymerase chain reaction using the amplification oligomers.

Another aspect of the invention relates to a kit for amplification of a stx1 gene and a stx2 gene. The kit includes the primer pair for amplification of a stx1 gene described above, and the primer pair for amplification of a stx2 gene described above.

Another aspect of the invention relates to a kit for detection of a stx1 gene and a stx2 gene. The kit includes any of the embodiments of the primer-probe sets for identification of stx1 as described above, in combination with any of the embodiments of the primer-probe sets for identification of stx2 as described above.

Another aspect of the invention relates to an amplification product for identification of a stx1 gene. This amplification product is produced by any of the embodiments of the method for identifying a stx1 gene as described above.

Another aspect of the invention relates to an amplification product for identification of a stx2 gene. This amplification product is produced by any of the embodiments of the method for identifying a stx2 gene as described above.

In another aspect, the present invention provides a method for identifying at least one of a stx1 gene and a stx2 gene in a sample. The method generally includes contacting the sample with a pair of stx1-specific amplification oligomers and a pair of stx2-specific amplification oligomers. Typically, each member of said stx1-specific and stx2-specific pairs of amplification oligomers has a length of from about 15 to about 25 contiguous nucleotides. In particular embodiments, the pair of stx1-specific amplification oligomers includes an oligomer pair selected from the following: (1-i) a first oligomer having at least 90%, at least 95% or 100% sequence identity to SEQ ID NO:30 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:31; (1-ii) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:1 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:2; (1-iii) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO: 8 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO: 9; (1-iv) a first oligomer having at least 90%, at least 95%, or 100% sequence identify to SEQ ID NO:12 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:13; and (1-v) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:19 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:20. In particular embodiments, the pair of stx2-specific amplification oligomers includes an oligomer pair selected from the following: (2-i) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:33 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:34; (2-ii) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:40 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:41; (2-iii) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO: 36 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO: 37; and (2-iv) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:47 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:48. The method further includes amplifying nucleic acid in the sample with the stx1-specific and/or stx2-specific pairs of amplification oligomers to obtain at least one amplification product, and determining the sequence of the at least one amplification product or detecting the at least one amplification product using a stx1-specific detection probe and a stx2-specific detection probe.

In some variations of a method as above for identifying at least one of stx1 and stx2 in a sample, each of the stx1-specific and stx2-specific detection probes is an oligomer having a length of from about 15 to about 30 contiguous oligomer residues. In particular embodiments, for the amplification oligomer pair of (1-i), the stx1-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:32; for the amplification oligomer pair of (1-ii), the stx1-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:5; for the amplification oligomer pair of (1-iii), the stx1-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO: 11; for the amplification oligomer pair of (1-iv), the stx1-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:14; or for the amplification oligomer pair of (1-v), the stx1-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:22. In particular embodiments, for the amplification oligomer pair of (2-i), the stx2-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:35; for the amplification oligomer pair of (2-ii), the stx2-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:42; for the amplification oligomer pair of (2-iii), the stx2-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO: 39; or for the amplification oligomer pair of (2-iv), the stx2-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:49.

In particular embodiments of a method as above for identifying at least one of stx1 and stx2 in a sample, the stx1-specific and stx2-specific pairs of amplification oligomers are selected from the following combinations of stx1-specific and stx2-specific oligomer pairs: (A) the amplification oligomer pairs of (1-i) and (2-i); (B) the amplification oligomer pairs of (1-i) and (2-ii); (C) the amplification oligomer pairs of (1-ii) and (2-i); (D) the amplification oligomer pairs of (1-iv) and (2-iv); (E) the amplification oligomer pairs of (1-v) and (2-i); and (F) the amplification oligomer pairs of (1-v) and (2-iv). In more specific variations of the method, for the combination of stx1-specific and stx2-specific oligomer pairs of (A), the stx1-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:32 and the stx2-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:35; for the combination of stx1-specific and stx2-specific oligomer pairs of (B), the stx1-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:32 and the stx2-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:42; for the combination of stx1-specific and stx2-specific oligomer pairs of (C), the stx1-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:5 and the stx2-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:35; for the combination of stx1-specific and stx2-specific oligomer pairs of (D), the stx1-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:14 and the stx2-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:49; for the combination of stx1-specific and stx2-specific oligomer pairs of (E), the stx1-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:22 and the stx2-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:35; or for the combination of stx1-specific and stx2-specific oligomer pairs of (F), the stx1-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:22 and the stx2-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:49.

In certain embodiments of a method as above for identifying at least one of stx1 and stx2 in a sample, the sample includes bacterial nucleic acid originating from Escherichia coli, Citrobacter freundii, Aeromononas hydrophila, Aeromononas caviae, or Enterobacter cloacae. In particular variations of the method, the bacterial nucleic acid originates from a strain of E. coli such as E. coli 0157:H7.

In some embodiments of the method for identifying at least one of stx1 and stx2 in a sample, the amplification step is performed using the polymerase chain reaction such as, for example, a real-time polymerase chain reaction.

In certain variations in which the detecting step includes detecting the at least one amplification product using the stx1-specific and stx2-specific detection probes, each of the detection probes is a fluorescence probe comprising a fluorescent dye compound. For example, each of the stx1-specific and stx2-specific detection probes may include a fluorescent dye compound and a non-fluorescent quenching dye compound.

In another aspect, the present invention provides a primer set for amplification of at least one of a stx1 gene and a stx2 gene in a sample. The primer set includes a pair of stx1-specific amplification oligomers and a pair of stx2-specific amplification oligomers, with each member of the pair typically having a length of from about 15 to about 25 contiguous nucleotides. In particular embodiments, the pair of stx1-specific amplification oligomers includes an oligomer pair selected from the following: (1-i) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:30 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:31; (1-ii) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:1 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:2; (1-iii) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO: 8 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO: 9; (1-iv) a first oligomer having at least 90%, at least 95%, or 100% sequence identify to SEQ ID NO:12 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:13; and (1-v) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:19 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:20. In particular embodiments, the pair of stx2-specific amplification oligomers includes an oligomer pair selected from the following: (2-i) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:33 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:34; (2-ii) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:40 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:41; (2-iii) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO: 36 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO: 37; and (2-iv) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:47 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:48.

In particular embodiments of a primer set as above, the stx1-specific and stx2-specific pairs of amplification oligomers are selected from the following combinations of stx1-specific and stx2-specific oligomer pairs: (A) the amplification oligomer pairs of (1-i) and (2-i); (B) the amplification oligomer pairs of (1-i) and (2-ii); (C) the amplification oligomer pairs of (1-ii) and (2-i); (D) the amplification oligomer pairs of (1-iv) and (2-iv); (E) the amplification oligomer pairs of (1-v) and (2-i); and (F) the amplification oligomer pairs of (1-v) and (2-iv).

In another aspect, the present invention provides a kit for amplification of at least one of a stx1 gene and a stx2 gene, the kit comprising a primer set as set forth above.

In yet another aspect, the present invention provides a primer-probe set for identification of at least one of a stx1 gene and a stx2 gene in a sample. The primer-probe set includes a pair of stx1-specific amplification oligomers and a pair of stx2-specific amplification oligomers, with each member of the stx1-specific and stx2-specific pairs of amplification oligomers typically having a length of from about 15 to about 25 contiguous nucleotides. The primer-probe set further includes a stx1-specific detection probe hybridizable to a stx1 gene region located between the regions of hybridization of the pair of stx1-specific amplification oligomers, and a stx2-specific detection probe hybridizable to a stx2 gene region located between the regions of hybridization of the pair of stx2-specific amplification oligomers. In particular embodiments, the pair of stx1-specific amplification oligomers includes an oligomer pair selected from the following: (1-i) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:30 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:31; (1-ii) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:1 and a second oligomer having at least

90%, at least 95%, or 100% sequence identity to SEQ ID NO:2; (1-iii) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO: 8 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO: 9; (1-iv) a first oligomer having at least 90%, at least 95%, or 100% sequence identify to SEQ ID NO:12 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:13; and (1-v) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:19 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:20. In particular embodiments, the pair of stx2-specific amplification oligomers includes an oligomer pair selected from the following: (2-i) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:33 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:34; (2-ii) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:40 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:41; (2-iii) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO: 36 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO: 37; and (2-iv) a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:47 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:48.

In certain variations of a primer-probe set as above each of the stx1-specific and stx2-specific detection probes is an oligomer having a length of from about 15 to about 30 or to about 40 contiguous oligomer residues. In particular embodiments, for the amplification oligomer pair of (1-i), the stx1-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:32; for the amplification oligomer pair of (1-ii), the stx1-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:5; for the amplification oligomer pair of (1-iii), the stx1-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO: 11; for the amplification oligomer pair of (1-iv), the stx1-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:14; or for the amplification oligomer pair of (1-v), the stx1-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:22. In particular embodiments, for the amplification oligomer pair of (2-i), the stx2-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:35; for the amplification oligomer pair of (2-ii), the stx2-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID

NO:42; for the amplification oligomer pair of (2-iii), the stx2-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO: 39; or for the amplification oligomer pair of (2-iv), the stx2-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:49.

In some variations of a primer-probe set as above, the stx1-specific and stx2-specific pairs of amplification oligomers are selected from the following combinations of stx1-specific and stx2-specific oligomer pairs: (A) the amplification oligomer pairs of (1-i) and (2-i); (B) the amplification oligomer pairs of (1-i) and (2-ii); (C) the amplification oligomer pairs of (1-ii) and (2-i); (D) the amplification oligomer pairs of (1-iv) and (2-iv); (E) the amplification oligomer pairs of (1-v) and (2-i); and (F) the amplification oligomer pairs of (1-v) and (2-iv). In some such variations, for the combination of stx1-specific and stx2-specific oligomer pairs of (A), the stx1-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:32 and the stx2-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:35; for the combination of stx1-specific and stx2-specific oligomer pairs of (B), the stx1-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:32 and the stx2-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:42; for the combination of stx1-specific and stx2-specific oligomer pairs of (C), the stx1-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:5 and the stx2-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:35; for the combination of stx1-specific and stx2-specific oligomer pairs of (D), the stx1-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:14 and the stx2-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:49; for the combination of stx1-specific and stx2-specific oligomer pairs of (E), the stx1-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:22 and the stx2-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:35; or for the combination of stx1-specific and stx2-specific oligomer pairs of (F), the stx1-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:22 and the stx2-specific probe has at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:49.

In certain embodiments of a primer-probe set as above, the primer-probe set further includes an internal control system for verifying reaction conditions. The internal control system typically comprises a control template polynucleotide, a pair of control amplification oligomers, and a control probe. In particular variations, the pair of control amplification oligomers includes a first oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:53 and a second oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:54. In some such variations, the control probe is an oligomer having at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO:55.

In still another aspect, the present invention provides a kit for identification of at least one of a stx1 gene and a stx2 gene, the kit comprising a primer-probe as set forth above.

DETAILED DESCRIPTION OF EMBODIMENTS

Definitions

To aid in understanding aspects of the disclosure, some terms used herein are described in more detail. All other scientific and technical terms used herein have the same meaning as commonly understood by those skilled in the relevant art, such as may be provided in Dictionary of Microbiology and Molecular Biology, 2nd ed. (Singleton et al., 1994, John Wiley & Sons, New York, NY), The Harper Collins Dictionary of Biology (Hale & Marham, 1991, Harper Perennial, New York, NY), and references cited herein. Unless mentioned otherwise, the techniques employed or contemplated herein are standard methods well known to a person of ordinary skill in the art of molecular biology.

It is to be noted that the term “a” or “an” entity refers to one or more of that entity; for example, “a nucleic acid,” is understood to represent one or more nucleic acids. As such, the terms “a” (or “an”), “one or more,” and “at least one” can be used interchangeably herein.

Sample. A “sample” or “specimen,” including “biological” or “clinical” samples may contain or may be suspected of containing STEC or components thereof, such as nucleic acids or fragments of nucleic acids. A sample may be a complex mixture of components. Samples include “biological samples” which include any tissue or material derived from a living or dead mammal or organism, including, for example, stool, blood, plasma, serum, blood cells, saliva, mucous and cerebrospinal fluid. Samples may also include samples of in vitro cell culture constituents including, for example, conditioned media resulting from the growth of cells and tissues in culture medium. The sample may be treated to chemically, physically or mechanically to disrupt tissue or cell structure to release intracellular nucleic acids into a solution which may contain enzymes, buffers, salts, detergents and the like, to prepare the sample for analysis. In one step of the methods described herein, a sample is provided that is suspected of containing at least one STEC target nucleic acid. Accordingly, this step excludes the physical step of obtaining the sample from a subject.

Polynucleotide. The term denotes a nucleic acid chain. Throughout this application, sequences of polynucleotides are expressed in a direction extending from the 5'-terminus to the 3'-terminus. Standard nucleic acids, e.g., DNA and RNA, are typically synthesized in the 3'-to-5' direction by the addition of nucleotides to the 5'-terminus of a growing nucleic acid.

Nucleotide. This is a subunit of a nucleic acid consisting of a phosphate group, a 5-carbon sugar and a nitrogenous base. The 5-carbon sugar found in RNA is ribose. In DNA, the 5-carbon sugar is 2'-deoxyribose. The term also includes analogs of RNA or DNA.

Nucleic acid. This refers to a multimeric compound comprising two or more covalently bonded nucleotides or nucleotide analogs having nitrogenous heterocyclic bases, or base analogs, where the nucleotides are linked together by phosphodiester bonds or other linkages to form a polynucleotide. Nucleic acids include RNA, DNA, or chimeric DNA-RNA polymers, and analogs thereof. A nucleic acid "backbone" may be made up of a variety of linkages, including one or more of sugar-phosphodiester linkages, peptide-nucleic acid bonds, phosphorothioate linkages, methylphosphonate linkages, or combinations thereof. Sugar moieties of the nucleic acid may be either ribose or deoxyribose, or similar compounds having known substitutions, e.g., 2'-methoxy substitutions and 2'-halide substitutions. Nitrogenous bases may be conventional bases (A, G, C, T, U), analogs thereof (e.g., inosine, 5-methylisocytosine, isoguanine; (The Biochemistry of the Nucleic Acids 5-36, Adams et al., ed., 11th ed., 1992, Abraham et al., 2007, BioTechniques 43: 617-24)), which include derivatives of purine or pyrimidine bases. Nucleic acids may include "abasic" residues in which the backbone does not include a nitrogenous base for one or more residues (see, for example US Pat. No. 5,585,481, which is incorporated herein by reference in entirety). Nucleic acids may include "locked nucleic acids" (LNA), in which one or more nucleotide monomers have a bicyclic furanose unit locked in an RNA-mimicking sugar conformation, which enhances hybridization affinity toward complementary sequences (Vester et al., 2004, Biochemistry 43(42):13233-41). Nucleic acids may include modified bases to alter the function or behaviour of the nucleic acid, e.g., addition of a 3'-terminal dideoxynucleotide to block additional nucleotides from being added to the nucleic acid. Synthetic methods for making nucleic acids in vitro are well known in the art although nucleic acids may be purified from natural sources using routine techniques.

Non-nucleotide unit. This is a unit that does not significantly participate in hybridization of a polymer. Such units do not, for example, participate in any significant hydrogen bonding with a nucleotide, and would exclude units having, as a component, one of the five canonical nucleotide bases or analogs thereof.

Target nucleic acid. This is a nucleic acid comprising a “target sequence” to be amplified. Target nucleic acids may be DNA or RNA and may be either single-stranded or double-stranded. In a preferred embodiment of the invention, the target nucleic acid is DNA. The target nucleic acid may include other sequences besides the target sequence that may be amplified. Typical target nucleic acids are the STEC genome or are derived from regions of the STEC genome.

Target sequence or **target nucleic acid sequence**. This term refers to the particular nucleotide sequence of the target nucleic acid that is to be amplified and/or detected. Where the target nucleic acid is originally single-stranded, the term “target sequence” will also refer to the sequence complementary to the target sequence as present in the target nucleic acid. Where the target nucleic acid is originally double-stranded, the term “target sequence” refers to both the sense (+) and antisense (-) strands. In choosing a target sequence, the skilled artisan will understand that a sequence should be chosen so as to distinguish between unrelated or closely related target nucleic acids. The terms “target(s) a sequence” or “target(s) a target nucleic acid” as used herein in reference to a region of STEC nucleic acid refer to a process whereby an oligonucleotide stably hybridizes to the target sequence in a manner that allows for amplification and/or detection as described herein. In one embodiment, the oligonucleotide is complementary to the targeted STEC nucleic acid sequence and contains no mismatches. In another embodiment, the oligonucleotide is complementary but contains 1; or 2; or 3; or 4; or 5 or more mismatches with the targeted STEC nucleic acid sequence. Preferably, the oligonucleotide that stably hybridizes to the STEC nucleic acid sequence includes at least 10 to 50 contiguous nucleotides complementary to the target sequence. It is understood that at least 10 and as many as 50 is an inclusive range such that 10, 50 and each whole number there between are included. The term "configured to target a sequence" as used herein means that the target hybridizing region of an oligonucleotide is designed to have a polynucleotide sequence that could target a sequence of the referenced STEC region. Such an amplification oligonucleotide is not limited to targeting that sequence only, but is rather useful in a composition, in a kit or in a method for targeting a STEC target nucleic acid, as is described herein. The term "configured to" denotes an actual arrangement of the polynucleotide sequence configuration of the amplification oligonucleotide target hybridizing sequence.

Fragment. This term, as used herein in reference to the STEC targeted nucleic acid sequence, refers to a piece of contiguous nucleic acid. In certain embodiments, the fragment includes contiguous nucleotides from a STEC target nucleic acid, wherein the number of contiguous nucleotides in the fragment is less than that for the entire STEC genome or a gene thereof.

Region. This term refers to a portion of a nucleic acid wherein said portion is smaller than the entire nucleic acid. For example, when the nucleic acid of reference is an oligonucleotide promoter, the term "region" may be used refer to the smaller promoter portion of the entire oligonucleotide. Similarly, and also as example only, when the nucleic acid is a target nucleic acid, the term "region" may be used to refer to a smaller area of the nucleic acid.

Oligonucleotide. This term may be used interchangeably with "oligomer and "oligo" and refers to a nucleic acid having generally less than 1,000 nucleotide (nt) residues. Preferably, an oligonucleotide is 10 nucleobases in length to 100 nucleobases in length. It is understood that these ranges are exemplary only, and it is further understood that an oligonucleotide can have a length that is any whole number included in the range from 10 to 100. Oligonucleotides may be purified from naturally occurring sources, or may be synthesized using any of a variety of well-known enzymatic or chemical methods. The term oligonucleotide does not denote any particular function to the reagent and rather is used generically to refer to all such reagents described herein. An oligonucleotide may have various functions. For example, it may function as a primer, a probe, a target capture oligomer or provide any other known function.

As used herein, an oligonucleotide having a nucleic acid sequence “comprising” or “consisting of” or “consisting essentially of” a sequence selected from a group of specific sequences means that the oligonucleotide has the ability to hybridize to a nucleic acid having the exact complement of one of the listed nucleic acid sequences of the group under stringent hybridization conditions. Though the hybridizing oligonucleotide and the target nucleic acid need not be 100% complementary to one another. An exact complement includes the corresponding DNA or RNA sequence.

Corresponds. As used herein, a nucleic acid “corresponds” to a specified nucleic acid if the nucleic acid is 100% identical or complementary to the specified nucleic acid.

Substantially corresponding to. As used herein, a nucleic acid “substantially corresponding to” a specified nucleic acid sequence, or its complement, means that the oligonucleotide is sufficiently similar to the reference nucleic acid sequence such that the oligonucleotide has similar hybridization properties to the reference nucleic acid sequence in that it would hybridize with the same target nucleic acid sequence under stringent hybridization conditions. Substantially corresponding nucleic acids vary by at least one nucleotide from the specified nucleic acid. This variation may be stated in terms of a percentage of sequence identity or complementarity between the nucleic acid and the specified nucleic acid (e.g., from less than 100% to about 80%). One skilled in the art will understand that the recited ranges include all whole and rational numbers of the range (e.g., 92%, 92.377%, etc).

Amplification oligomer. An “amplification oligomer,” which may also be called an “amplification oligonucleotide,” is an oligomer, at least the 3'-end of which is complementary to part of a target sequence, and which hybridizes to the target sequence and participates in a nucleic acid amplification reaction. A primer may hybridize specifically to a single species of target nucleic acid. Alternatively, a primer may hybridize to a region of a plurality of target nucleic acids wherein the region is substantially conserved amongst the species. An example of an amplification oligomer is a “primer” that hybridizes to a target nucleic acid and contains a 3'-OH end that is extended by a polymerase in an amplification process. Amplification oligomers include oligonucleotides that comprise a 3'-target hybridizing region and a 5'-region that is not configured for hybridizing the target nucleic acids. Examples of such 5'-regions include, but are not limited to, promoter sequences, tag sequences, barcode sequences and the like. The 3'- and 5'-regions may be directly linked (e.g., a phosphorothioate linkage) or indirectly linked (e.g., a 9-carbon linker). An example of an amplification oligomer comprising a 3' target hybridizing segment and a 5'-non-target-hybridizing segment is a "promoter-based amplification oligomer," which comprises a 5'-promoter sequence for initiating transcription by an appropriate polymerase. Size ranges for amplification oligonucleotides include ranges which include target hybridizing regions that are about 10 nucleotides to about 70 nucleotides long, including all whole numbers between 10 and 70. An amplification oligomer may optionally include modified nucleotides or analogs that are not complementary to a target nucleic acid in a strict A:T/U, G:C sense. Such modified nucleotides or analogs are herein considered mismatched to their corresponding target sequence.

Amplification. This refers to any known procedure for obtaining multiple copies of a target nucleic acid sequence or its complement or fragments thereof. The multiple copies may be referred to as amplicons or amplification products. Known amplification methods include both thermal cycling and isothermal amplification methods. Transcription mediated amplification (TMA), polymerase chain reaction (PCR), replicase-mediated amplification, ligase chain reaction (LCR), strand-displacement amplification (SDA), and transcription-mediated or transcription-associated amplification are non-limiting examples of nucleic acid amplification methods (see for example US Pat. Nos. 4,868,105; 5,124,246; 5,130,238; 5,399,491; 5,437,990; 5,554,516; and 7,374,885; and PCT Pub. Nos. WO 88/01302; WO 88/10315 and WO 95/03430 (TMA); US Pat. No. 4,786,600 (RCA); US Pat. No. 5,427,930 and US Pat. No. 5,516,663 (LCR); and US Pat. No. 5,422,252; US Pat. No. 5,547,861; and US 5,648,211 (SDA), each of which is incorporated herein by reference in entirety). PCR is the preferred amplification method, and is well known in the art. Briefly, PCR amplification uses a DNA polymerase, pairs of primers, and thermal cycling to synthesize multiple copies of two complementary strands from dsDNA or from a cDNA (see for example, US Pat. Nos. 4,683,195, 4,683,202, and 4,800,159, each of which is incorporated herein by reference in entirety).

Real-time amplification. As used herein, the term "real-time amplification" refers to amplification of target nucleic acid that is monitored by real-time detection means. Real-time PCR amplification includes a method and reagents for performing what is commonly referred to as Taqman® PCR (see for example, Holland et al., PNAS 88 (16): 7276–7280 (1991); and Livak et al, U.S. Pat. No. 6,030,787, each of which is incorporated herein by reference in entirety).

Amplicon. This term, which is used interchangeably with the term "amplification product," refers to the nucleic acid molecule generated during an amplification procedure that is complementary or homologous to a sequence contained within the target sequence. These terms can be used to refer to a single strand amplification product, a double strand amplification product or one of the strands of a double strand amplification product.

Probe. A probe, also known as a "detection probe" or "detection oligonucleotide" are terms referring to a nucleic acid oligomer that hybridizes specifically to a target sequence in a nucleic acid, or in an amplified nucleic acid, under conditions that promote hybridization to allow detection of the target sequence or amplified nucleic acid. Probe lengths are preferably in the range from 10 nucleobases to 100 nucleobases, inclusive of all whole numbers therein. Detection may either be direct (e.g., a probe hybridized directly to its target sequence) or indirect (e.g., a probe linked to its target via an intermediate molecular structure). Probes may be DNA, RNA, analogs thereof or combinations thereof and they may be labeled or unlabeled. A probe may comprise target-specific sequences and other sequences that contribute to the three-dimensional conformation of the probe (see for example, US Pat. Nos. 5,118,801; 5,312,728; 6,849,412; 6,835,542; 6,534,274; and 6,361,945; and US Pub. No. 20060068417, each of which are incorporated herein by reference in entirety). Detection probes may comprises a 2'-O-methoxy residue which can result in a higher signal being obtained. In general the term "TaqMan® probe" refers to oligonucleotides that contain a fluorescent dye, typically on the 5' base, and a non-fluorescent quenching dye (quencher), typically on the 3' base. When irradiated, the excited fluorescent dye transfers energy to the nearby quenching dye molecule rather than fluorescing, resulting in a non-fluorescent substrate. During amplification, the exonuclease activity of the polymerase cleaves the TaqMan® probe to separate the fluorophore from the quencher, thereby allowing an unquenched signal to be emitted from the fluorophore as an indicator of amplification.

Label. As used herein, a "label" refers to a moiety or compound joined directly or indirectly to a probe that is detected or leads to a detectable signal. Direct labeling can occur through bonds or interactions that link the label to the probe, including covalent bonds or non-covalent interactions, e.g. hydrogen bonds, hydrophobic and ionic interactions, or formation of chelates or coordination complexes. Indirect labeling can occur through use of a bridging moiety or "linker" such as a binding pair member, an antibody or additional oligomer, which is either directly or indirectly labeled, and which may amplify the detectable signal. Labels may be detectable in a homogeneous assay in which bound labeled probe in a mixture exhibits a detectable change different from that of an unbound labeled probe. A "homogeneous detectable label" can be detected without physically removing bound from unbound forms of the label or labeled probe (see for example, US Pat. Nos. 5,118,801, 5,283,174, 5,312,728, 5,656,207, and 5,658,737, each of which are incorporated herein by reference in entirety). Labels include any detectable moiety, such as a radionuclide, ligand (such as biotin, avidin), enzyme or enzyme substrate, reactive group, or chromophore (such as a dye, particle, or bead that imparts detectable color), luminescent compound (such as bioluminescent, phosphorescent, or chemiluminescent labels), or fluorophore. Common labels used for TaqMan® detection probes include a fluorophore and a quencher. Exemplary fluorophores include FAM, SYBR® Green, VIC, JOE, NED, Cy3, ROX, Texas Red and Cy5 dyes (all well known in the art and readily available from numerous commercial sources). Exemplary quenchers include BHQ, TAMRA and DABCLY (all well known in the art and readily available from numerous commercial sources). Synthesis and methods of attaching labels to nucleic acids and detecting labels are well known (see for example, Sambrook et al., Molecular Cloning, A Laboratory Manual, 2nd ed. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989), Chapter 10; US Pat. Nos. 5,658,737, 5,656,207, 5,547,842, 5,283,174, and 4,581,333, each of which is incorporated herein by reference in entirety). More than one label, and more than one type of label, may be present on a particular probe, or detection may use a mixture of probes in which each probe is labeled with a compound that produces a different detectable signal (see for example, US Pat. Nos. 6,180,340 and 6,350,579, each of which is incorporated herein by reference in entirety).

Stable. "Stable" or "stable for detection" means that the temperature of a reaction mixture is at least 2°C below the melting temperature of a nucleic acid duplex.

Capture oligonucleotide. As used herein, a "capture oligonucleotide," "target capture oligonucleotide" or "capture probe" refers to a nucleic acid oligomer that specifically hybridizes to a target sequence in a target nucleic acid by standard base pairing and joins to a binding partner on an immobilized probe to capture the target nucleic acid to a support. One example of a capture oligomer includes an oligonucleotide comprising two binding regions: a target hybridizing region and an immobilized probe-binding region. These two regions may be part of a single contiguous nucleic acid molecule or may be two different oligomers joined together by one or more linkers. A capture oligomer may have a target hybridizing sequence that substantially corresponds to a specific target sequence. Alternatively, a capture oligomer may have a target hybridizing sequence that includes random or non-random poly-GU, poly-GT, or poly U sequences to bind non-specifically to a plurality of nucleic acids, including the target nucleic acid. (PCT Pub No. WO 2008/016988, incorporated herein by reference in entirety). Capture oligomers may include at least one 2' O-methoxy linkage. The immobilized probe binding region can be a nucleic acid sequence, referred to as a tail. Tails include a substantially homopolymeric tail of about 10 to 40 nucleotides (for example. $T_{0-4}A_{10-40}$), or of about 14 to 33 nt (e.g., T_3A_{14} to T_3A_{30}), that bind to a complementary immobilized sequence attached to the support particle or support matrix. Thus, a non-limiting example of preferred nucleic acid tails can in some embodiments include $T_{0-4}A_{10-36}$ sequences. Another example of a capture oligomer comprises two regions, a target hybridizing sequence and a binding pair member that is not a nucleic acid sequence (see PCT Pub No. WO 2008/016988 at page 3, lines 2-8 for examples of non-nucleotide binding pairs).

Immobilized oligonucleotide. As used herein, an “immobilized oligonucleotide,” “immobilized probe” or “immobilized nucleic acid” refers to a nucleic acid binding partner that joins a capture oligomer to a support, directly or indirectly. An immobilized probe joined to a support facilitates separation of a capture probe bound target from unbound material in a sample. One embodiment of an immobilized probe is an oligomer joined to a support that facilitates separation of bound target sequence from unbound material in a sample. Supports may include known materials, such as matrices and particles free in solution, which may be made of nitrocellulose, nylon, glass, polyacrylate, mixed polymers, polystyrene, silane, polypropylene, metal, or other compositions, of which one embodiment is magnetically attractable particles. Supports may be monodisperse magnetic spheres (e.g., uniform size ± 5%), to which an immobilized probe is joined directly (via covalent linkage, chelation, or ionic interaction), or indirectly (via one or more linkers), where the linkage or interaction between the probe and support is stable during hybridization conditions.

Complementary. The term “complementary” means that nucleotide sequences of similar regions of two single-stranded nucleic acids, or to different regions of the same single-stranded nucleic acid have a nucleotide base composition that allow the single-stranded regions to hybridize together in a stable double-stranded hydrogen-bonded region under stringent hybridization or amplification conditions. Sequences that hybridize to each other may be completely complementary or partially complementary to the intended target sequence by standard nucleic acid base pairing (e.g. G:C, A:T or A:U pairing). The term "sufficiently complementary" refers to a contiguous sequence that is capable of hybridizing to another sequence by hydrogen bonding between a series of complementary bases, which may be complementary at each position in the sequence by standard base pairing or may contain one or more residues that are not complementary by standard A:T/U and G:C pairing, or are modified nucleotides such as abasic residues, modified nucleotides or nucleotide analogs. Sufficiently complementary contiguous sequences typically are at least 80% complementary to a sequence to which an oligomer is intended to specifically hybridize (a percentage complementarity range includes all whole and rational numbers of the range). Sequences that are "sufficiently complementary" allow stable hybridization of a nucleic acid oligomer with its target sequence under appropriate hybridization conditions, even if the sequences are not completely complementary.

Preferentially hybridize. The term "preferentially hybridize" means that under stringent hybridization assay conditions, an oligonucleotide hybridizes to its target sequences, or amplicons thereof, to form a stable oligonucleotide: target sequence hybrid, while at the same time, formation of a stable hybrid between an oligonucleotide and a non-target sequence is minimized. For example, a probe oligonucleotide preferentially hybridizes to a target sequence or amplicon thereof to a sufficiently greater extent than to a non-target sequence, to enable detection of the target sequence and amplicon thereof. Appropriate hybridization conditions are well known in the art for probe, amplification, target capture and other oligonucleotides, and may be predicted based on sequence composition, or can be determined by using routine testing methods (see for example Sambrook et al., Molecular Cloning, A Laboratory Manual, 2nd ed. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989) at §§ 1.90-1.91, 7.37-7.57, 9.47-9.51 and 11.47-11.57, particularly §§ 9.50-9.51, 11.12-11.13, 11.45-11.47 and 11.55-11.57, incorporated herein by reference in entirety).

Nucleic acid hybrid. The term "nucleic acid hybrid" or "hybrid" or "duplex" refers to a nucleic acid structure containing a double-stranded, hydrogen-bonded region that is at least substantially complementary one to the other, and wherein the duplex is sufficiently stable under stringent hybridization conditions to be detected by means including, but not limited to, chemiluminescent or fluorescent light detection, autoradiography, or gel electrophoresis.

Such hybrids may comprise RNA:RNA, RNA:DNA, or DNA:DNA duplex molecules.

Sample preparation. This refers to any steps or methods that treat a sample for subsequent amplification and/or detection of STEC nucleic acids present in the sample. The target nucleic acid may be a minority component in the sample. Sample preparation may include any known method of isolating or concentrating components, such as viruses or nucleic acids using standard microbiology methods. Sample preparation may include physical or mechanical disruption and/or chemical lysis of cellular components to release intracellular components into a substantially aqueous or organic phase and removal of debris, such as by using filtration, centrifugation or adsorption. Sample preparation may include use of a nucleic acid oligonucleotide that selectively or non-specifically captures a target nucleic acid and separates it from other sample components (see for example US Patent No. 6,110,678 and PCT Pub. No. WO 2008/016988, each of which is incorporated herein by reference in entirety).

Separating, purifying, isolating. These terms mean that one or more components of a sample are removed or separated from other sample components. Sample components include target nucleic acids usually in a generally aqueous solution phase, which may also include cellular fragments, proteins, carbohydrates, lipids, and other nucleic acids. Separating or purifying removes at least 70%, or at least 80%, or at least 95% of the target nucleic acid from other sample components. Ranges of %-purity include all whole and rational numbers of the range.

Specificity. The term “specificity,” in the context of an amplification system, is used herein to refer to the characteristic of an amplification system which describes its ability to distinguish between target and non-target sequences dependent on sequence and assay conditions. In terms of nucleic acid amplification, “specificity” generally refers to the ratio of the number of specific amplicons produced to the number of side-products (for example, the signal-to-noise ratio).

Sensitivity. The term “sensitivity” is used herein to refer to the precision with which a nucleic acid amplification reaction can be detected or quantitated. The sensitivity of an amplification reaction is generally a measure of the smallest copy number of the target nucleic acid that can be reliably detected in the amplification system, and will depend, for example, on the detection assay being employed, and the specificity of the amplification reaction which is characterized, for example, by the ratio of specific amplicons to side-products.

Overview

Nucleic acid oligomer sequences are disclosed that may serve as primers and/or detection probes for amplification and/or detection of nucleic acids derived from Shiga toxin-producing E. coli (STEC) or other organisms that produce Shiga toxins. The nucleic acids derived from of Shiga toxin genes may be detected in a sample by using methods of in vitro nucleic acid amplification such as PCR (for example. Taqman® PCR), or transcription-associated amplification - such as TMA or NASBA. Amplification oligomers are configured to specifically hybridize to a Shiga toxin target sequence and generate an amplicon therefrom. Detection probes hybridize specifically to at least a portion of the amplified sequence, either after completion of or during the amplification process. The methods further may use oligonucleotide sequences that serve as capture probes for processing a sample by capturing the target Shiga toxin nucleic acid and separating it from other sample components (see US Pat. Nos. 6,110,678, 6,280,952 and 6,534,273, which are incorporated herein by reference in entirety).

Methods disclosed herein can be used to detect nucleic acids derived from Shiga toxin genes present in samples from or derived from animals and humans.

Compositions disclosed herein include amplification oligomers that can be used to specifically amplify selected nucleic acid sequences present in Shiga toxin genes, and optionally nucleic acid probes for detecting the amplified sequences.

The disclosed nucleic acid sequences and methods are useful for amplifying and detecting nucleic acids from Shiga toxin genes or derived from bacteria present in a sample in a relatively short time so that diagnosis can be made quickly and so that effective treatment can be initiated to limit the spread of the bacteria. Thus, the methods and compositions disclosed herein respond to a need for rapid, sensitive, and specific testing of clinical samples that may contain bacteria with genes encoding Shiga toxins.

The disclosed probe sequences may be used as primers, and the disclosed primers may be used as probes. The same is true for the disclosed probe hybridization regions and primer hybridization regions of a given target gene. Thus, the probe hybridization regions disclosed herein may be used as primer hybridization regions. Likewise, primer hybridization regions disclosed herein may be used as probe hybridization regions.

Oligonucleotides for amplifying a Shiga toxin producing E. coli (STEC) target typically comprise at least two amplification oligomers. Some embodiments of the invention may utilise, three, four, five, or even six or ten or more amplification oligomers in, for example, multiplex amplification assays. Thus, by way of example, oligonucleotides for amplifying a Shiga toxin target gene may comprise one, two, three, four, or five or more forward amplification primers and one, two, three, four, or five or more reverse amplification primers. In one embodiment, at least one of the amplification oligomers is configured to specifically hybridize to a region within a target sequence corresponding to the stx1 gene. In another embodiment, at least one of the amplification oligomers is configured to specifically hybridize to a region within a target sequence corresponding to the stx2 gene. In one embodiment, at least two amplification oligomers are used, wherein the amplification oligomers are respectively configured to specifically hybridize to regions within a target sequence selected from the group consisting of stx1 gene and stx2 gene in order to generate an amplicon that can be subsequently detected. Suitably, the amplicon is detectable using a detection probe. Suitably, the amplicon is from 50 to 210 nucleotides in length, including all whole numbers between 50 and 210 that are not explicitly listed here.

In one embodiment, at least one of the amplification oligomers is configured to specifically hybridize to a region within a target sequence of STEC corresponding to nucleotides 2924490-2925716 of GenBank accession number BA000007, GI number 47118301. In one embodiment, at least one of the amplification oligomers is configured to specifically hybridize to a region within a target sequence of STEC corresponding to nucleotides 1266965-1268205 of GenBank accession number BA000007, GI number 47118301. In another embodiment, at least two amplification oligomers are used, wherein the amplification oligomers are configured to specifically hybridize to regions within a target sequence of STEC corresponding to nucleotides 2924490-2925719 of GenBank accession number BA000007, GI number 47118301, in order to generate an amplicon that can be subsequently detected. In another embodiment, at least two amplification oligomers are used, wherein the amplification oligomers are configured to specifically hybridize to regions within a target sequence of STEC corresponding to nucleotides 1266965-1268205 of GenBank accession number BA000007, GI number 47118301, in order to generate an amplicon that can be subsequently detected.

In one embodiment, at least one of the amplification oligomers is configured to specifically hybridize to a region within a target sequence of STEC corresponding to nucleotides 101-246, 346-506, 399-614, 535-663, 568-727, 595-793, 775-898, and 1071-1200 of SEQ ID NO:56. In one embodiment, at least one of the amplification oligomers is configured to specifically hybridize to a region within a target sequence of STEC corresponding to nucleotides 23-131, 387-498, 519-697, 654-748, and 841-1029 of SEQ ID NO:57. In another embodiment, at least two amplification oligomers are used, wherein the amplification oligomers are configured to specifically hybridize to regions within a target sequence of STEC corresponding to nucleotides 101-246, 346-506, 399-614, 535-663, 568-727, 595-793, 775-898, and 1071-1200 of SEQ ID NO:56 in order to generate an amplicon that can be subsequently detected. In another embodiment, at least two amplification oligomers are used, wherein the amplification oligomers are configured to specifically hybridize to regions within a target sequence of STEC corresponding to nucleotides 23-131, 387-498, 519-697, 654-748, and 841-1029 of SEQ ID NO:57 in order to generate an amplicon that can be subsequently detected.

Oligomers for amplifying and/or detecting Shiga toxin target genes (amplification oligomers) include oligonucleotide sequences selected from the group consisting of SEQ ID NOS: 1 to 49 or a combination of two or more thereof. According to one embodiment, at least one first amplification oligomer comprises, consists of or consists essentially of a target hybridizing sequence selected from the group consisting of SEQ ID NOS: 1, 8, 12, 15, 19, 23, 27, 30, 33, 36, 40, 44, or 47 or a combination of two or more thereof. According to one embodiment, at least one second amplification oligomer comprises, consists of consists essentially of a target hybridizing sequence selected from the group consisting of SEQ ID NOS: 2, 6, 9, 13, 16, 20, 24, 28, 31, 34, 37, 41, 45, or 48 or a combination of two or more thereof. Although these sequences are shown as DNA sequences, equivalent RNA sequences can be readily derived by the person skilled in the art and are to be considered as falling within the definition of "oligomers." In addition, complementary sequences of DNA and RNA and reverse complementary sequences can be readily derived by the skilled person. It is therefore to be understood that a description of any individual sequence of DNA, for example, encompasses its complement, its reverse complement and equivalent RNA sequences.

The methods for detecting nucleic acid of a Shiga toxin gene optionally include a detecting step that uses at least one probe that binds specifically to the amplified STEC product (RNA or DNA amplicon, preferably DNA amplicon). In one embodiment, one or more detection probes are configured to detect a sequence in a region corresponding to part of SEQ ID NO:56. In one embodiment, one or more detection probes are configured to detect a sequence in a region corresponding to part of SEQ ID NO:57. In one embodiment, one or more detection probes is individually configured to detect a sequence in a region corresponding to nucleotides 101-246, 346-506, 399-614, 535-663, 568-727, 595-793, 775-898, and 1071-1200 of SEQ ID NO:56. In another embodiment, one or more detection probes are configured to detect a sequence in a region corresponding to nucleotides 23-131, 387-498, 519-697, 654-748, and 841-1029 of SEQ ID NO:57. Probes for the specific detection of STEC sequences include oligomers selected from the group consisting of SEQ ID Nos. 3, 4, 5, 7, 10, 11, 14, 17, 18, 21, 22, 25, 26, 29, 32, 35, 38, 39, 42, 43, 46, 49, or a combination of two or more thereof.

Oligonucleotides for amplifying and detecting the STEC target typically comprise at least two amplification oligomers and at least one probe. Some embodiments of the invention may utilise four, five, six, seven, eight or more amplification oligomers and two, three, four, five or even six or more probes. Thus, by way of example, oligonucleotides for amplifying and detecting a Shiga toxin gene target may comprise two or three or more forward amplification primers together with two or three or more reverse amplification primers together with two, three, four, five or even six or more probes.

Assays for detection of a Shiga toxin gene may include an internal control (IC) nucleic acid that is amplified and detected by using IC-specific primers and probe in the same reaction mixtures used for amplification and detection of a region of a Shiga toxin gene. Amplification and detection of the IC-specific sequence demonstrates that assay reagents and conditions were properly used even when a signal specific for a Shiga toxin gene is not detected for a tested sample (i.e., negative samples). The IC may be used as an internal calibrator for the assay that provides a quantitative result. The IC may be a randomized sequence derived from a naturally occurring source bacterium that does not harbor a Shiga toxin gene. An example of an IC-specific primer-probe set is SEQ ID NO:53 (forward), SEQ ID NO:54 (reverse) and SEQ ID NO:55 (probe).

Combinations of amplification oligomers specific for identification of a Shiga toxin gene are therefore contemplated. Each member of the combination may have at least 90% sequence identity to 100% sequence identity to any of the SEQ ID NOs of the amplification oligomers described herein. For greater clarity, each member of a combination of amplification oligomers may have at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity with the SEQ ID NOs of the amplification oligomers described herein.

According to one embodiment, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:1 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:7. In particular variations, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:1 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:2. In other variations, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:1 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:6.

According to another embodiment, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:8 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:11. In particular variations, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:8 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:9.

According to another embodiment, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence as with at least 90% sequence identity to SEQ ID NO:12 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:13 or SEQ ID NO:14. In particular variations, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence as with at least 90% sequence identity to SEQ ID NO:12 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:13.

According to another embodiment, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:15 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:16, SEQ ID NO:17, or SEQ ID NO:18. In particular variations, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:15 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:16.

According to one embodiment, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:19 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:20, SEQ ID NO:21, or SEQ ID NO:22. In particular variations, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:19 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:20.

According to another embodiment, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:23 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26. In particular variations, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:23 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:24.

According to one embodiment, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:27 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:28 or SEQ ID NO:29. In particular variations, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:27 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:28.

According to one embodiment, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:30 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:31 or SEQ ID NO:32. In particular variations, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:30 is used in combination with at least one second

amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:31.

According to one embodiment, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:33 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:34 or SEQ ID NO:35. In particular variations, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:33 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:34.

According to one embodiment, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:36 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:37, SEQ ID NO:38, or SEQ ID NO:39. In particular variations, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:36 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:37.

According to one embodiment, at least one first amplification oligomer comprising, consisting or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:40 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:41, SEQ ID NO:42, or SEQ ID NO:43. In particular variations, at least one first amplification oligomer comprising, consisting or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:40 is used in combination with at least one second

amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:41.

According to one embodiment, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:44 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:45 or SEQ ID NO:46. In particular variations, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:44 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:45.

According to one embodiment, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:47 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:48 or SEQ ID NO:49. In particular variations, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:47 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:48.

According to one embodiment, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:50 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:51 or SEQ ID NO:52. In particular variations, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:50 is used in combination with at least one second

amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:51.

According to one embodiment, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:53 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:54 or SEQ ID NO:55. In particular variations, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:53 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:54.

Combinations of oligomers and probes that can be used for the amplification and detection of multiple STEC targets are also disclosed.

In one embodiment, two amplification oligomers and one probe are configured to specifically hybridize to a region within a target sequence of a Shiga toxin gene corresponding to nucleotides 101-246, 346-506, 399-614, 535-663, 568-727, 595-793, 775-898 or 1071-1200 of SEQ ID NO:56. In one embodiment, two amplification oligomers and one probe are configured to specifically hybridize to a region within a target sequence of a Shiga toxin gene corresponding to nucleotides 23-131, 387-498, 519-697, 654-748 or 841-1029 of SEQ ID NO:57.

Each probe in the primer-probe combinations for identification of a Shiga toxin gene described herein may have at least 90% sequence identity to 100% sequence identity to any of the SEQ ID NOs of the probes described herein. For greater clarity, each probe in a primer-probe combination may have at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity with the SEQ ID Nos of the probes described herein.

In one embodiment, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to

SEQ ID NO:1 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:2, and is used in combination with at least one probe comprising, consisting of of consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:3, SEQ ID NO:4, or SEQ ID NO:5; or a sequence with at least 90% sequence identity to SEQ ID NO:1 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:6, and is used in combination with at least one probe comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:4 or SEQ ID NO:7.

In one embodiment, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:8 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:9, and is used in combination with at least one probe comprising, consisting of, of consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:10 or SEQ ID NO:11.

In one embodiment, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:12 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:13, and is used in combination with at least one probe comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:14.

In one embodiment, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:15 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:16, and is used in combination with at least one probe comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:17 or SEQ ID NO:18.

In one embodiment, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:19 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:20, and is used in combination with at least one probe comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:21 or SEQ ID NO:22.

In one embodiment, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:23 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:24, and is used in combination with at least one probe comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:25 or SEQ ID NO:26.

In one embodiment, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:27 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:28, and is used in combination with at least one probe comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:29.

In one embodiment, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:30 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:31 is used in combination with at least one probe comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:32.

In one embodiment, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:33 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:34, and is used in combination with at least one probe comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:35.

In one embodiment, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:36 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:37, and is used in combination with at least one probe comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:38 or SEQ ID NO:39.

In one embodiment, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:40 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:41, and is used in combination with at least one probe comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:42 or SEQ ID NO:43.

In one embodiment, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:44 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:45, and is used in combination with at least one probe comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:46.

In one embodiment, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:47 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:48, and is used in combination with at least one probe comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:49.

In one embodiment, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:50 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:51, and is used in combination with at least one probe comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:52.

In one embodiment, at least one first amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:53 is used in combination with at least one second amplification oligomer comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:54, and is used in combination with at least one probe comprising, consisting of, or consisting essentially of a target hybridizing sequence with at least 90% sequence identity to SEQ ID NO:55.

In another embodiment, two different sets of primers and probes configured to hybridize STEC target regions are combined. For convenience, combinations of STEC regions are provided in Table 1. In the description below, the parenthesized letters correspond to STEC target sequences as follows: For a target sequence of a STEC gene corresponding to SEQ ID NO:56, 101-246 (A), 346-506 (B), 399-614 (C), 535-663 (D), 568-727 (E), 595-793 (F), 775-898 (G) and1071-1200 (H); and for a target sequence of a STEC gene corresponding to SEQ ID NO:57, 23-131 (I), 387-498 (J), 519-697 (K), 654-748 (L) and 841-1029 (M).

TABLE 1

	A	B	C	D	E	F	G	H	I	J	K	L	M
A	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ	AK	AL	AM
B	BA	BB	BC	BD	BE	BF	BG	BH	BI	BJ	BK	BL	BM
C	CA	CB	CC	CD	CE	CF	CG	CH	CI	CJ	CK	CL	CM
D	DA	DB	DC	DD	DE	DF	DG	DH	DI	DJ	DK	DL	DM
E	EA	EB	EC	ED	EE	EF	EG	EH	EI	EJ	EK	EL	EM
F	FA	FB	FC	FD	FE	FF	FG	FH	FI	FJ	FK	FL	FM
G	GA	GB	GC	GD	GE	GF	GG	GH	GI	GJ	GK	GL	GM
H	HA	HB	HC	HD	HE	HF	HG	HH	HI	HJ	HK	HL	HM
I	IA	IB	IC	ID	IE	IF	IG	IH	II	IJ	IK	IL	IM
J	JA	JB	JC	JD	JE	JF	JG	JH	JI	JJ	JK	JL	JM
K	KA	KB	KC	KD	KE	KF	KG	KH	KI	KJ	KK	KL	KM
L	LA	LB	LC	LD	LE	LF	LG	LH	LI	LJ	LK	LL	LM
M	MA	MB	MC	MD	ME	MF	MG	MH	MI	MJ	MK	ML	MM

Thus in Table 1, an embodiment of two different sets of primers and probes configured to hybridize STEC target sequences shown as BM, means that one set of primers and probe are configured to hybridize STEC target sequence B (corresponding to nucleotides 346-506 of SEQ ID NO:56) and the second set of primers and probe are configured to hybridize STEC target sequence M (corresponding to nucleotides 841-1029 of SEQ ID NO:57). Embodiments of two different sets of primers and probes configured to hybridize STEC target sequences shown in Table 1 using the same letter (e.g. AA) means that the first and second primer/probe sets are both configured to target the same region (in this example, a region corresponding to nucleotides 101-246 of SEQ ID NO:56) but the first primer/probe set is different than the second primer probe set.

In one embodiment, a multiplex comprises at least two amplification oligomers and probe combinations wherein the first amplification oligomers and probe combination comprises, consists, or consists essentially of at least three target hybridizing sequence selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:5 and wherein the second amplification oligomers and probe combination comprises, consists, or consists essentially of: at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:12, SEQ ID NO:13, and

SEQ ID NO:14; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, and SEQ ID NO:18; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, and SEQ ID NO:22; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:27, SEQ ID NO:28, and SEQ ID NO:29; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:33, SEQ ID NO:34,and SEQ ID NO:35; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:28, and SEQ ID NO:39; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, and SEQ ID NO:43; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:44, SEQ ID NO:45, and sequences with at least 90% sequence identity to SEQ ID NO:46; or at least three target hybridizing sequences selected from the group consisting of SEQ ID NO:47, SEQ ID NO:48, and SEQ ID NO:49.

In one embodiment, a multiplex comprises at least two amplification oligomers and probe combinations wherein the first amplification oligomers and probe combination comprises, consists, or consists essentially of at least three target hybridizing sequence selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:7 and wherein the second amplification oligomers and probe combination comprises, consists, or consists essentially of: at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:12, SEQ ID NO:13, and SEQ ID NO:14; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, and SEQ ID NO:18; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, and SEQ ID NO:22; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:27, SEQ ID NO:28, and SEQ ID NO:29; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:33, SEQ ID NO:34, and SEQ ID NO:35; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, and SEQ ID NO:39; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, and SEQ ID NO:43; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:44, SEQ ID NO:45, and SEQ ID NO:46; or at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:47, SEQ ID NO:48, and SEQ ID NO:49.

In one embodiment, a multiplex comprises at least two amplification oligomers and probe combinations wherein the first amplification oligomers and probe combination comprises, consists, or consists essentially of at least three target hybridizing sequence selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:12, SEQ ID NO:13, and SEQ ID NO:14 and wherein the second amplification oligomers and probe combination comprises, consists, or consists essentially of: at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, and SEQ ID NO:18; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, and SEQ ID NO:22; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:27, SEQ ID NO:28, and SEQ ID NO:29; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:33, SEQ ID NO:34, and SEQ ID NO:35; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, and SEQ ID NO:39; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, and SEQ ID NO:43; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:44, SEQ ID NO:45, and SEQ ID NO:46; or at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:47, SEQ ID NO:48, and SEQ ID NO:49.

In one embodiment, a multiplex comprises at least two amplification oligomers and probe combinations wherein the first amplification oligomers and probe combination comprises, consists, or consists essentially of at least three target hybridizing sequence selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, and SEQ ID NO:18 and wherein the second amplification oligomers and probe combination comprises, consists, or consists essentially of: at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, and SEQ ID NO:22; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:27, SEQ ID NO:28, and SEQ ID NO:29; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:33, SEQ ID NO:34, and SEQ ID NO:35; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, and SEQ ID NO:39; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, and SEQ ID NO:43; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:44, SEQ ID NO:45, and SEQ ID NO:46; or at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:47, SEQ ID NO:48, and SEQ ID NO:49.

In one embodiment, a multiplex comprises at least two amplification oligomers and probe combinations wherein the first amplification oligomers and probe combination comprises, consists, or consists essentially of at least three target hybridizing sequence selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, and SEQ ID NO:22 and wherein the second amplification oligomers and probe combination comprises, consists, or consists essentially of: at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:27, SEQ ID NO:28, and SEQ ID NO:29; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, and SEQ ID NO:39; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, and SEQ ID NO:43; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:44, SEQ ID NO:45, and SEQ ID NO:46; or at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:47, SEQ ID NO:48, and SEQ ID NO:49.

In one embodiment, a multiplex comprises at least two amplification oligomers and probe combinations wherein the first amplification oligomers and probe combination comprises, consists, or consists essentially of at least three target hybridizing sequence selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26 and wherein the second amplification oligomers and probe combination comprises, consists, or consists essentially of: at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:27, SEQ ID NO:28, and SEQ ID NO:29; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:33, SEQ ID NO:34, and SEQ ID NO:35; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, and SEQ ID NO:39; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, and SEQ ID NO:43; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:44, SEQ ID NO:45, and SEQ ID NO:46; or at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:47, SEQ ID NO:48, and SEQ ID NO:49.

In one embodiment, a multiplex comprises at least two amplification oligomers and probe combinations wherein the first amplification oligomers and probe combination comprises, consists, or consists essentially of at least three target hybridizing sequence selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:27, SEQ ID NO:28, and SEQ ID NO:29 and wherein the second amplification oligomers and probe combination comprises, consists, or consists essentially of: at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:27, SEQ ID NO:28, and SEQ ID NO:29; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:33, SEQ ID NO:34, and SEQ ID NO:35; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, and SEQ ID NO:39; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, and SEQ ID NO:43; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:44, SEQ ID NO:45, and SEQ ID NO:46; or at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:47, SEQ ID NO:48, and SEQ ID NO:49.

In one embodiment, a multiplex comprises at least two amplification oligomers and probe combinations wherein the first amplification oligomers and probe combination comprises, consists, or consists essentially of at least three target hybridizing sequence selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:27, SEQ ID NO:28, and SEQ ID NO:29 and wherein the second amplification oligomers and probe combination comprises, consists, or consists essentially of: at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:33, SEQ ID NO:34, and SEQ ID NO:35; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, and SEQ ID NO:39; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, and SEQ ID NO:43; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:44, SEQ ID NO:45, and SEQ ID NO:46; or at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:47, SEQ ID NO:48, and SEQ ID NO:49.

In one embodiment, a multiplex comprises at least two amplification oligomers and probe combinations wherein the first amplification oligomers and probe combination comprises, consists, or consists essentially of at least three target hybridizing sequence selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:33, SEQ ID NO:34, and SEQ ID NO:35 and wherein the second amplification oligomers and probe combination comprises, consists, or consists essentially of: at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, and SEQ ID NO:39; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, and SEQ ID NO:43; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:44, SEQ ID NO:45, and SEQ ID NO:46; or at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:47, SEQ ID NO:48, and SEQ ID NO:49.

In one embodiment, a multiplex comprisies at least two amplification oligomers and probe combinations wherein the first amplification oligomers and probe combination comprises, consists, or consists essentially of at least three target hybridizing sequence selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, and SEQ ID NO:39 and wherein the second amplification oligomers and probe combination comprises, consists, or consists essentially of: at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, and SEQ ID NO:43; at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:44, SEQ ID NO:45, and SEQ ID NO:46; or at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:47, SEQ ID NO:48, and SEQ ID NO:49.

In one embodiment, a multiplex comprises at least two amplification oligomers and probe combinations wherein the first amplification oligomers and probe combination comprises, consists, or consists essentially of at least three target hybridizing sequence selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, and SEQ ID NO:43 and wherein the second amplification oligomers and probe combination comprises, consists, or consists essentially of: at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:44, SEQ ID NO:45, and SEQ ID NO:46; or at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:47, SEQ ID NO:48, and SEQ ID NO:49.

In one embodiment, a multiplex comprises at least two amplification oligomers and probe combinations wherein the first amplification oligomers and probe combination comprises, consists, or consists essentially of at least three target hybridizing sequence selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:44, SEQ ID NO:45, and SEQ ID NO:46 and wherein the second amplification oligomers and probe combination comprises, consists, or consists essentially of at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:4, SEQ ID NO:48, and SEQ ID NO:49.

In another embodiment, a multiplex comprising at least two amplification oligomers and probe combinations further includes a third amplification oligomers and probe combination comprising internal control (IC) amplification primers and probe. In one embodiment, the third amplification oligomer and probe set comprises, consists, or consists essentially of: at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:50, SEQ ID NO:51, and SEQ ID NO:52; or at least three target hybridizing sequences selected from the group consisting of sequences with at least 90% sequence identity to SEQ ID NO:53, SEQ ID NO:54, and SEQ ID NO:55.

The oligomers for use in the methods described herein are suited for preparation of kits. Such a kit may comprise containers, each with one or more of the various oligomers optionally together with one or more of the reagents or enzymes required to perform the methods described herein. The components of the kit may be supplied in concentrated form. A set of instructions for using the components of the kit will also typically be included. Where the kit comprises combinations of oligomers then the individual oligomers may be provided in individual form, with appropriate instructions for mixing same, or combinations thereof that are ready mixed.

The detection of an amplicon from a sample using primers of the instant invention is indicative of the presence of a Shiga toxin gene in the sample. The detection of an amplicon from a sample using primers and probes of the instant invention is indicative of the presence of STEC in the sample. The detection of amplified target sequences characteristic of a Shiga toxin gene in a biological sample from an individual is indicative of infection with a bacterium containing a Shiga toxin. The detection of an amplicon in a human biological sample using the primer pairs described herein is indicative of an infection of the human with a bacterium containing a Shiga toxin. The detection of an amplicon in a human biological sample using the primer and probe combinations described herein is also indicative of such an infection in that human.

EXAMPLES

Example 1: Exemplary Nucleic Acid Sequences

This example provides exemplary sequences that are useful with the present invention. Table 2 does not limit the scope of the invention. Sequences are presented according to Tables 1 through 6 of Appendix 2 of the World Intellectual Property Organization (WIPO) Handbook on Industrial Property Information and Documentation, Standard ST.25 (1998).

The sequences in Table 2 are associated with sequence identifiers (SEQ ID NOs) and the function of each sequence (forward primer, reverse primer or probe) is also indicated as well as the target.

Table 2: Exemplary nucleic acid sequences

SEQ ID NO:	**Sequence 5′ → 3′**	**Function**	**Target**
1	TGACAGTAGCTATACCACGT	Forward primer	*stx1*
2	AGTGTTGTACGAAATCCCCT	Reverse primer	*stx1*
3	ATCAGTCGTACGGGGATGCAGA	Probe	*stx1*
4	ACAGCGTGTTGCAGGGATCAGTC	Probe	*stx1*
5	ATGCAGATAAATCGCCATTCGTTGAC	Probe	*stx1*
6	CTATGCGACATTAAATCCAGAT	Reverse primer	*stx1*
7	TAGTCAACGAATGGCGATTTATCTGCA	Probe	*stx1*
8	AGACGTATGTAGATTCGCTG	Forward primer	*stx1*
9	TGGATCTATCCCTCTGACAT	Reverse primer	*stx1*
10	ATGTCATTCGCTCTGCAATAGGTACT	Probe	*stx1*
11	ATCATCAGTAAAGACGTACCTCCTGAT	Probe	*stx1*
12	GCTGATTTTTCACATGTTACCT	Forward primer	*stx1*
13	CTATGCGACATTAAATCCAGAT	Reverse primer	*stx1*
14	TTGTCTGGTGACAGTAGCTATACCAC	Probe	*stx1*
15	AGGGGATTTCGTACAACACT	Forward primer	*stx1*
16	AATTCAGTATTAATGCCACGCT	Reverse primer	*stx1*
17	ACACGAACAGAGTCTTGTCCATGATA	Probe	*stx1*
18	ATGATCTCAGTGGGCGTTCTTATGTA	Probe	*stx1*
19	AGAGCGATGTTACGGTTTGT	Forward primer	*stx1*

20	ATCAACATCTTCAGCAGTCATT	Reverse primer	*stx1*
21	AGCTGAAGCTTTACGTTTTCGGCAAAT	Probe	*stx1*
22	CATAAGAACGCCCACTGAGATCATC	Probe	*stx1*
23	GAAGCTTTACGTTTTCGGCA	Forward primer	*stx1*
24	GAACAGAGTCTTGTCCATGA	Reverse primer	*stx1*
25	CTACTCAACCTTCCCCAGTTCAATG	Probe	*stx1*
26	AGAGGGGATTTCGTACAACACTGGA	Probe	*stx1*
27	GTGGCATTAATACTGAATTGTC	Forward primer	*stx1*
28	ACAATATTTTATTGTGCGTAATCC	Reverse primer	*stx1*
29	ATCATGCATCGCGAGTTGCCAGAA	Probe	*stx1*
30	CGATACCTTTACAGTTAAAGTG	Forward primer	*stx1*
31	TCCATTATGACAGGCATTAGTT	Reverse primer	*stx1*
32	ATGGAATCTTCAGTCTCTTCTTCTCAGT	Probe	*stx1*
33	GGGTACTGTGCCTGTTAC	Forward primer	*stx2*
34	GTCCGTATACTATTTAACGAAG	Reverse primer	*stx2*
35	TTCTTCGGTATCCTATTCCCGGGA	Probe	*stx2*
36	CATGACAACGGACAGCAG	Forward primer	*stx2*
37	CTCCATTAACGCCAGATATG	Reverse primer	*stx2*
38	ACCAGTGAGTGACGACTGATTTGCAT	Probe	*stx2*
39	ATACCACTCTGCAACGTGTCGCA	Probe	*stx2*
40	CAGAGATGCATCCAGAGC	Forward primer	*stx2*
41	CCGGAAGCACATTGCTGA	Reverse primer	*stx2*
42	CAGAGAGAATTTCGTCAGGCACTG	Probe	*stx2*
43	ACAGGAGCAGTTTCAGACAGTGC	Probe	*stx2*
44	GGACCTCACTCTGAACTG	Forward primer	*stx2*
45	TATTATTAAAGGATATTCTCCCCA	Reverse primer	*stx2*
46	TCAGCAATGTGCTTCCGGAGTATC	Probe	*stx2*
47	GAATGTCAGATAAGTGGCGA	Forward primer	*stx2*
48	CCGCCATTGCATTAACAGAA	Reverse primer	*stx2*
49	TGCAAATAAAACCGCCATAAACATCTTCT	Probe	*stx2*
50	CAGGAGCGTAGGAATACCATC	Forward primer	Internal control
51	GGGCTAACTGTGAAGATTCAATAG	Reverse primer	Internal control
52	TCACTGATTCAAGCACGTTAGAAGGCCA	Probe	Internal control
53	GAGTTGGTAAACAGATCATGC	Forward primer	Internal control
54	GACTTGTGGCCTTCTAACG	Reverse primer	Internal control
55	TATCGAGCACAGGAGCGTAGGAATA	Probe	Internal control

SEQ ID NOs: 56 and 57 (below) are the sequences of stx1 and stx2, respectively, of a strain of E. coli O157:H7.

SEQ ID NO:56, Escherichia coli O157:H7 str. Sakai DNA, complete genome GenBank accession number BA000007, GI number 47118301 bases 2924490-2925719

aatatgaaaataattattttagagtgctaactttttctttgttatcttttcagttaatgtggttgcgaaggaa
tttaccttagacttctcgactgcaaagacgtatgtagattcgctgaatgtcattcgctctgcaataggtactcca
ttacagactatttcatcaggaggtacgtctttactgatgattgatagtggcacaggggataatttgtttgcagtt
gatgtcagagggatagatccagaggaagggcggtttaataatctacggcttattgttgaacgaaataatttatat
gtgacaggatttgttaacaggacaaataatgtttttatcgctttgctgattttcacatgttacctttccaggt
acaacagcggttacattgtctggtgacagtagctataccacgttacagcgtgttgcagggatcagtcgtacgggg
atgcagataaatcgccattcgttgactacttcttatctggatttaatgtcgcatagtggaacctcactgacgcag
tctgtggcaagagcgatgttacggtttgttactgtgacagctgaagctttacgttttcggcaaatacagaggga
tttcgtacaacactggatgatctcagtgggcgttcttatgtaatgactgctgaagatgttgatcttacattgaac
tggggaaggttgagtagtgtcctgcctgattatcatggacaagactctgttcgtgtaggaagaatttcttttgga
agcattaatgcaattctgggaagcgtggcattaatactgaattgtcatcatcatgcatcgcgagttgccagaatg
gcatctgatgagtttccttctatgtgtccggcagatggaagagtccgtgggattacgcacaataaaatattgtgg
gattcatccactctgggggcaattctgatgcgcagaactattagcagttgagggggtaaaatgaaaaaaacatta
ttaatagctgcatcgctttcatttttttcagcaagtgcgctggcgacgcctgattgtgtaactggaaaggtggag
tatacaaaatataatgatgacgatacctttacagttaaagtgggtgataaagaattatttaccaacagatggaat
cttcagtctcttcttctcagtgcgcaaattacggggatgactgtaaccattaaaactaatgcctgtcataatgga
gggggattcagcgaagttatttttcgttga

SEQ ID NO:57, Escherichia coli O157:H7 str. Sakai DNA, complete genome GenBank accession number BA000007, GI number 47118301 bases 1266965-1268205

atgaagtgtatattatttaaatgggtactgtgcctgttactgggtttttcttcggtatcctattcccgggagttt
acgatagacttttcgacccaacaaagttatgtctcttcgttaaatagtatacggacagagatatcgacccctctt
gaacatatatctcaggggaccacatcggtgtctgttattaaccacaccccaccgggcagttattttgctgtggat
atacgagggcttgatgtctatcaggcgcgttttgaccatcttcgtctgattattgagcaaaataatttatatgtg
gccgggttcgttaatacggcaacaaatactttctaccgttttttcagattttacacatatatcagtgcccggtgtg
acaacggtttccatgacaacggacagcagttataccactctgcaacgtgtcgcagcgctggaacgttccggaatg
caaatcagtcgtcactcactggtttcatcatatctggcgttaatggagttcagtggtaatacaatgaccagagat
gcatccagagcagttctgcgttttgtcactgtcacagcagaagccttacgcttcaggcagatacagagagaattt
cgtcaggcactgtctgaaactgctcctgtgtatacgatgacgccgggagacgtggacctcactctgaactggggg
cgaatcagcaatgtgcttccggagtatcggggagaggatggtgtcagagtggggagaatatcctttaataatata
tcagcgatactggggactgtggccgttatactgaattgccatcatcagggggcgcgttctgttcgcgccgtgaat
gaagagagtcaaccagaatgtcagataactggcgacaggcctgttataaaaataaacaatacattatgggaaagt
aatacagctgcagcgtttctgaacagaaagtcacagttttatatacaacgggtaaataaaggagttaagcatga
agaagatgtttatggcggttttatttgcattagcttctgttaatgcaatggcggcggattgtgctaaaggtaaaa
ttgagttttccaagtataatgaggatgacacatttacagtgaaggttgacgggaaagaatactggaccagtcgct
ggaatctgcaaccgttactgcaaagtgctcagttgacaggaatgactgtcacaatcaaatccagtacctgtgaat
caggctccggatttgctgaagtgcagtttaataatgactga

Example 2: Preparation of DNA

All strains of STEC, including various (O157 and non-O157 strains) were obtained from the STEC Center at Michigan State University). All bacteria were cultured by streaking onto agar plates, incubating overnight at 37°C, and transferring to 4°C the next morning. Individual colonies were selected, inoculated into 3 mL of Luria broth, incubated overnight at 37°C, and transferred to 4°C the next morning. A 20 µL aliquot of the inoculated broth was serially diluted (dilution range was 10-10^{10}) to determine colony counts. A volume of 100 µL of each serial dilution was spread on an agar plate, incubated overnight at 37°C, and the colonies were counted the next day. DNA was isolated from the bacteria using two different methods. The first method used the NucleiSENS® easyMag® and associated reagents available and sold by bioMérieux (Durham, NC). In the second method, the bacteria were placed in Cary-Blair medium with 0.1 mm glass beads and vortexed for 10 minutes with the tube lying horizontally.

Example 3: Initial Testing of Shiga Toxin Gene 1 and Shiga Toxin Gene 2 Primers

In this example, primers specific for the Shiga toxin gene 1 (stx1) and Shiga toxin gene 2 (stx2) were evaluated to determine if they would amplify the target gene and not amplify non-target genes. The primers were evaluated using the following bacteria: Shiga Toxin producing E. coli (STEC), Shigella flexneri, Salmonella enterica sv. Typhimurium, Campylobacter jejuni, Shigella sonnei, and Salmonella enterica sv. Enteritidis. The primer combinations, along with the expected size in base pairs (bp), are shown in Table 3, below. Combination numbers P1-P9 target stx1 and combination numbers P10-P14 target stx2.

Table 3: Amplification Primer Pair Combinations

Primer Pair Combination No.	Forward SEQ ID NO:	Reverse SEQ ID NO:	Expected Amplicon Size (bp)
P1	1	2	217
P2	1	6	108
P3	8	9	146
P4	12	13	162
P5	15	16	200
P6	19	20	129
P7	23	24	161
P8	27	28	124
P9	30	31	130
P10	33	34	109
P11	36	37	115
P12	40	41	181
P13	44	45	95

Primer Pair Combination No.	**Forward SEQ ID NO:**	**Reverse SEQ ID NO:**	**Expected Amplicon Size (bp)**
P14	47	48	191

The primers were evaluated at a concentration of 250 μM, using various concentrations of bacteria. Five μL volumes of solutions containing bacteria at various concentrations or diliutions were added to amplification mixtures. Bacterial targets other than Salmonella Enteritidis and Shigella sonnei were diluted to provide 1, 10, 10^2, 10^3, 10^5 copies of bacterial DNA per μL in 5μL. Salmonella Enteritidis and Shigella sonnei were provided as 5μL of bacterial stocks. The amplification mixtures were subjected to amplification by PCR using an ABI 2720 thermocycler with the following conditions: 1 cycle of 95°C for 10 minutes; 50 cycles of 95°C for 15 seconds and 55°C for 35 seconds. The amplification product was detected by capillary electrophoresis using Qiagen QIAxcel cartridges. The results of this experiment are outlined in Table 4 below. The comment field provides notes regarding interpretation of the results. It was later determined that the Shigella flexneri genomic DNA was contaminated with STEC genomic DNA and therefore, a cross-reaction with Shigella flexneri as template resulting in the expected amplicon size for a stx gene target in Table 4 does not represent an adverse result. On the basis of these results, primer pair combination P3 (SEQ ID NOs: 8 and 9) targeting the stx1 gene and primer pair combination P11 (SEQ ID NOs: 36 and 37) targeting stx2 gene, were among the primer pairs selected for further testing.

Table 4: Detection of E. coli Amplification Products

Primer Pair Combination	**Expected Amplicon Size (bp)**	**Lowest Concentration of *E. coli* Detected (copies/μL)**	**Comments**
P1	217	4.75	Detected an *E. coli* band at 189bp & an *S. sonnei* band at 644 bp
P2	108	5.23	Detected a ~85 bp band for *Salmonella* species
P3	146	8.2	cross-reaction with *Shigella flexneri* at 151 bp (7.18ng/μL)
P4	162	8.5	
P5	200	7.79	cross-reaction with *Shigella flexneri* evidenced by band at 195 bp (6.18ng/μL) and primer dimer (~50 bp) for *C. coli, S. sonnei*
P6	129	8.34	Detected an *S. sonnei* band at 669bp

Primer Pair Combination	**Expected Amplicon Size (bp)**	**Lowest Concentration of *E. coli* Detected (copies/µL)**	**Comments**
P7	161	996	Poor sensitivity
P8	124	1.94	
P9	130	5.08	*E. coli* band detected at 122bp
P10	109	4.79	
P11	115	7.49	Cross-reaction with *Shigella flexneri* (e.g., band at 115 bp (5.25ng/µL)) was later determined to be STEC genomic DNA contamination of the *Shigella flexneri* stock. Primer dimers were observed (~48 bps) in all samples except the negative control. *S. enterica* also detected with bands at 387 bp and 474 bp.
P12	181	7.34	*S. enterica* detected with bands at 419 bp and 528 bp. *S. flexneri* detected with band at 287 bp. *S. sonnei* detected with bands at 129 and 326 bp (6.74ng/µL). The negative control has a band at 80bp band.
P13	95	4.18	S. typhimurium 44bp band (primer dimer)
P14	191	929	Poor sensitivity

Example 4: Specificity Testing of Select Shiga Toxin Gene 1 and Shiga Toxin Gene 2 Primers and Probes

In this example, primers and probes targeting the Shiga toxin gene 1(stx1) and Shiga toxin gene 2 (stx2) were evaluated for cross-reactivity to the bacteria listed in Table 5, below. The primer and probe combinations are shown in Table 6, below, where "PPC#" indicates the primer/probe combination number. PPC1 to PPC5 target the stx1 gene and PPC6 to PPC8 target the stx2 gene.

Table 5: Bacteria Tested

Bacteria	**ATCC No.**
Salmonella enterica sv Typhi	6539
Salmonella enterica sv Newport	6962
Salmonella enterica sv Heidelberg	8326
Salmonella enterica sv Typhimurium	14028
Salmonella bongori	43975
Salmonella enterica sv Typhimurium	BAA-189
Salmonella enterica sv Paratyphi B	8759
Salmonella enterica sv Typhimurium	BAA-191
Salmonella enterica sv Newport	27869

Bacteria	**ATCC No.**
Salmonella enterica sv Enteritidis	6961
Salmonella enterica sv Typhimurium	BAA-215
Salmonella enterica sv Enteritidis	4931
Salmonella enterica sv Typhimurium	19585
Salmonella enterica sv Enteritidis	BAA-708
Salmonella enterica sv Enteritidis	BAA-1045
Salmonella enterica sv Montevideo	BAA-710
Salmonella enterica subspecies *enterica sv* Javiana	BAA-1593
Salmonella enterica subspecies *enterica sv Typhimiurium*	BAA-1603
Shigella sonnei	9290
Shigella flexneri	12022
Shigella sonnei	29930
Shigella sonnei	11060
Shigella flexneri serotype *6*	12025
Shigella dysenteriae	29027
Shigella sonnei	25931
Shigella dysenteriae	29026
Shigella boydii serotype *2*	25930
Shigella flexneri serotype *3*	11836
Shigella boydii serotype *1*	9207
Shigella flexneri serotype *2a*	25875
Shigella sonnei	29030
Shigella flexneri serotype *1a*	9199
Campylobacter jejuni ssp. jejuni	22428
Campylobacter fetus ssp. fetus	33246
Campylobacter jejuni ssp. jejuni	3329
Campylobacter coli	43474

Table 6: Primer and Probe Combinations

Primer Pair-Probe Combination No.	**Forward SEQ ID NO:**	**Reverse SEQ ID NO:**	**Probe SEQ ID NO:**
PPC1	1	2	5
PPC2	8	9	10
PPC3	8	9	11
PPC4	19	20	21
PPC5	23	24	25
PPC6	33	34	35
PPC7	36	37	38
PPC8	36	37	39

The primers and probes were evaluated at 200 µM, using various concentrations of bacteria. The probes for primer/probe combinations PPC1 to PPC5 were labeled with CalOrange and BHQ1 (BioSearch Technologies, Novato, CA). The probes for primer/probe combinations PPC6 to PPC8 were labeled with FAM and BHQ1 (BioSearch Technologies, Novato, CA). Nucleic acids were extracted from 5 µL solutions containing bacteria at either 10^5 CFU/mL or 10^{-4} dilution of ATCC stock solutions by providing 200 µL of dilution to an easyMAG system (BioMerieux, Durham, NC, Nucleisens EasyMAG), and eluting into 110 µL volume.

Eluted nucleic acids were amplified by PCR on a Cepheid SmartCycler® (Sunnyvale, CA), using the following protocol: 1 cycle of 95°C for 10 minutes; 5 cycles of 95°C for 30 seconds and 55°C for 60 seconds; and 45 cycles of 95°C for 10 seconds and 55°C for 60 seconds. The positive control was E. coli strain EDL 933 genomic DNA, (Item # 700927D, Lot# 3791888, 1 × 10^5 copies/μL diluted 1:10 before use. Water was used as a negative control. The controls verified that the assay operates as intended. All of the stx1 primer/probe combinations were found to cross-react with Shigella dysenteriae, ATCC # 29026. This was an expected result because this strain contains a Shiga Toxin gene similar to stx1. On the other hand, none of the stx2 primer/probe combinations were found to cross-react with any of the bacteria. Primer/probe combination PPC5 had false positives with the positive control. This is due to "bleed-over" of the detection label into alternative detection channels and is based on the optics of the system. There was also some erroneous optics in primer/probe combinations 1-5, but only with the S. dysenteriae where it would test positive in the CalOrange (TET) channel generating erroneous curves in the FAM channel due to the high signal generated in the TET channel. The results of this example indicate that the primer-probe combinations targeted to stx1 can be used to identify a specific strain of Shigella dysenteriae (29026) which is known to contain a Shiga toxin. It was not previously known whether this strain carries the stx1 gene or the stx2 gene and it now appears that this strain carries the stx1-like gene. This experiment also demonstrates proper functioning of the selected probes with the primer pairs.

Example 5: Specificity and Reliability of Shiga Toxin 1, Shiga Toxin 2, and Internal Control Triplex Primer-Probe Combinations

In this example, seven primer and probe combinations for Shiga toxin gene 1(stx1) and Shiga toxin gene 2 (stx2) were evaluated in a triplex reaction with an internal control primer and probe combination. Twenty-one strains of E. coli were evaluated. The 21 strains are EcMLST numbers TW14960, TW02302, TW07927, TW07814, TW09153, TW08569, TW07926, TW00975, TW07960, TW04863, TW05149, TW07596, TW07989, TW07591, TW07700, TW07931, TW07947, TW08101, TW06296, TW00971, and TW09183. Each EcMLST number represents a strain of pathogenic E. coli characterized by multi-locus sequence typing. The EcMLST database can be accessed via the internet or by contacting Microbial Evolution Laboratory, 165 National Food Safety and Toxicology Center, Michigan State University, East Lansing, MI 48824. The other bacteria investigated include

Salmonella bongori, Salmonella enterica subsp enterica ser Typhi, Salmonella enterica subsp enterica ser Paratyphi, Salmonella enterica subsp enterica ser Typhimurium (6 strains), Salmonella enterica subsp enterica ser Enteritidis (4 strains), Salmonella enterica subsp enterica ser Newport (2 strains), Salmonella enterica subsp enterica ser Heidelberg, Salmonella enterica subsp enterica ser Javiana, Salmonella enterica subsp enterica ser Montevideo, Shigella boydii (2 strains), Shigella dysenteriae (2 strains), Shigella flexneri (5 strains), Shigella sonnei (6 strains), Campylobacter jejuni subsp jejuni (7 strains), Campylobacter coli (4 strains), Campylobacter lari (2 strains), Campylobacter upsaliensis (2 strains), Campylobacter hyointestinalis, Campylobacter fetus sub fetus, Campylobacter helveticus, Campylobacter gracilis, Campylobacter curvus, Campylobacter sputorum biovar sputorum, Campylobacter sputorum biovar faecalis, Campylobacter rectus, Campylobacter showae, and Campylobacter mucosalis. The primer and probe combinations tested are shown in Table 7, below. "PPC#" indicates the primer/probe combination number with reference to the codes shown in Table 6 above (PPC1 to PPC8). PPC9 has a forward primer of SEQ ID NO: 19, a reverse primer of SEQ ID NO: 20, and a probe of SEQ ID NO: 22.

Table 7: Primer and Probe Combinations

MIXTURE#	***stx1*** **Primer Probe Set**	***stx2*** **Primer Probe Set**
M1	PPC1	PPC7
M2	PPC2	PPC7
M3	PPC2	PPC8
M4	PPC3	PPC7
M5	PPC3	PPC8
M6	PPC9	PPC7
M7	PPC9	PPC8

Each primer and probe was evaluated at 200 μM. The probes for stx1 were labeled with CALflour0560 and BHQ1, the probes for stx2 were labeled with FAM and BHQ1, and the probes for the IC were labeled with Quasar670 and BHQ2. Primer/probe combinations were evaluated using 10^4 CFU/μL and 10^3 CFU/μl of extracted E. coli nucleic acids and all other bacteria were extracted and their resultant nucleic acids tested at various concentrations. Each reaction mixture also contained a primer pair for amplification of an internal control polynucleotide and a probe. This primer and probe set is used as an internal control to verify if any PCR inhibitors are present in the sample and determine that PCR amplification conditions are operating properly. A 5 μL volume of each nucleic acid extract of bacteria was amplified using PCR using the Cepheid SmartCycler® system with the following protocol: 1 cycle of 95°C for 60 seconds; 5 cycles of 95°C for 30 seconds and 55°C for 60 seconds; and 45 cycles of 95°C for 10 seconds and 55°C for 60 seconds. No detections were observed for any of the strains that do not contain the stx1 or sxt2 genes. Shown below in Table 8 are the cycle threshold (Ct) values (wherein lower numbers indicate more efficient amplification) for the 12 strains containing the stx2 gene in PCR reactions. Table entries with "**0**" indicate an amplification failure. In these cases, the average Ct values are calculated accordingly, with the "0" entries are omitted from the calculation. It can be seen that the mean Ct is the lowest for mixture M5 which contains primer probe combinations PPC3 (SEQ ID NOs: 8, 9 and 11) and PPC8 (SEQ ID Nos: 36, 37 and 39). It is also seen that mixture M5 did not have any amplification failures. This example provides an indication that mixture M5 is efficient for amplification with respect to stx2. Additional experiments (not shown) have confirmed that mixture M5 is also effective for amplification of stx1.

Table 8: Cycle Threshold Values Determined for Dual Target Mixtures in Amplification Reactions with Samples Containing stx2

Sample ID	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**
1 at 10^4 CFU/mL	33	33.3	33.4	33.2	33	33.5	33
1 at 10^3 CFU/mL	36	36.8	36.4	39.2	35.7	38.1	36.6
2 at 10^4 CFU/mL	32.3	32.3	32.1	32.1	32.2	32.9	32.3
2 at 10^3 CFU/mL	34.9	35.1	37.8	35.7	35.3	36.5	35.7
3 at 10^4 CFU/mL	33.7	33.3	33.4	34.1	33.1	33.6	33.3
3 at 10^3 CFU/mL	35.7	**0**	37.2	37.3	35.7	36.4	37.6
4 at 10^4 CFU/mL	32.2	32	32.2	32.4	33.1	32.2	32.1
4 at 10^3 CFU/mL	35.1	35.3	35	35.6	34.7	35.2	35.4
5 at 10^4 CFU/mL	32.3	32.9	33.3	32.2	32.2	32.8	32.7
5 at 10^3 CFU/mL	36.1	38.6	35.8	37.9	34.9	35.5	36.5
6 at 10^4 CFU/mL	32.7	32.7	33.5	32.5	32.4	32.9	32.9
6 at 10^3 CFU/mL	36.2	36.8	35.5	35.1	35	35.6	36.3
7 at 10^4 CFU/mL	32.2	33.5	33.4	32.4	31.9	33.4	32.4
7 at 10^3 CFU/mL	36.2	36.2	**0**	38.4	35.8	**0**	**0**
8 at 10^4 CFU/mL	31.2	31.8	31.6	31.4	31.7	32.5	31.4

8 at 10^3 CFU/mL	34.3	34.8	34.5	34.6	35.1	34.7	34.3
9 at 10^4 CFU/mL	30.3	31	31.8	31.1	30.7	31.3	31.2
9 at 10^3 CFU/mL	33.6	34.1	33.7	34.2	34.3	33.4	34.5
10 at 10^4 CFU/mL	32.1	32.4	32.7	32.3	32	33.1	31.9
10 at 10^3 CFU/mL	35.5	36.5	39.1	35.7	36.5	35.7	38.2
11 at 10^4 CFU/mL	33.1	33.2	33.6	33.3	32.4	33.4	33.3
11 at 10^3 CFU/mL	37.1	35.4	37.2	36.1	36.2	36.5	36.7
12 at 10^4 CFU/mL	31.5	31.1	30.9	31.2	30.9	31.8	31.1
12 at 10^3 CFU/mL	34.3	35.7	34.7	34.5	34.1	34.2	34.2
Avg at 10^4 CFU/mL	**32.2**	**32.5**	**32.7**	**32.4**	**32.1**	**32.8**	**32.3**
Avg at 10^3 CFU/mL	**35.4**	**35.9**	**36.1**	**36.2**	**35.3**	**35.6**	**36**
Positive Control	27.2	27.4	28	27.8	27.2	26.8	27.8

Results for stx1 and IC are not shown. It is notable that mixtures M2, M3, M4, M6, and M7 missed some detections at the lower of the two concentrations tested and/or had false positives for the stx1 detection. Mixture 1 had 3 instances of false positive results in the IC (CY5) channel exhibiting non-specific interaction of the oligonucleotides in that specific mix allowing for the Quasar 670 signal to be detected in the absence of any IC template. More specifically, mixture M2 missed a detection of stx2 at 10^3 CFU/mL of strain TW 07814. M3 missed a detection of stx1 at 10^3 CFU/mL in strain TW07960, missed detection of both stx1 and stx2 at 10^3 CFU/mL in strain TW4863, and had a false positive detection of stx1 in strain TW07931. Mixture M4 missed a detection of stx1 at 10^3 CFU/mL in strain TW04863, TW07947, and in strain TW08101 and had a false positive detection of stx1 in strain TW07927 . Mixture M6 missed a detection of stx1 at 10^3 CFU/mL in strain TW07947 and missed a detection of stx2 at 10^3 in strain TW04863. These results indicate that mixtures M2, M3, M4, M6 and M7 are not as reliable as some of the other mixtures. Additional experiments (not shown) have also established that an internal control primer-probe set comprising SEQ ID NO: 53 (forward), SEQ ID NO: 54 (reverse), and SEQ ID NO: 55 (probe) works well with mixture M5. However, the skilled person will recognize that other internal control systems which include a template polynucleotide and a primer/probe set can be used to replace this internal control system. The skilled person will understand that such appropriate replacements of internal control systems can be identified without undue experimentation.

Example 6: Optimization of stx2/stx1 Primer-Probe Combinations

Based on data generated in, e.g., reactivity and limit of detection (LoD) studies in stool and clinical samples, three different stx2/stx1 primer-probe combinations (see Table 9, below) were selected for further evaluation and optimization using STEC genomic DNA as template.

Table 9: stx2/stx1 Primer-Probe Combinations

Combination Designation (*stx2/stx1*)	**SEQ ID NOS:**	**Name (Description)**	**5′ Modification**	**3′ Modification**
1a/9a	33	*stx2* #1F (forward primer)	None	None
	34	*stx2* #1R (reverse primer)	None	None
	35	*stx2* #1a (Internal Quencher BHQ-1) (detection probe)	FAM	Spacer C3
	30	*stx1* #9F (forward primer)	None	None
	31	*stx1* #9R (reverse primer)	None	None
	32	*stx1* #9a (detection probe)	CalO 560	BHQ-1
3a/9a	40	*stx2* #3F (forward primer)	None	None
	41	*stx2* #3R (reverse primer)	None	None
	42	*stx2* #3a (Internal Quencher BHQ-1)	FAM	Spacer C3

Combination Designation (*stx2/stx1*)	**SEQ ID NOS:**	**Name (Description)**	**5′ Modification**	**3′ Modification**
		(detection probe)		
	30	*stx1* #9F (forward primer)	None	None
	31	*stx1* #9R (reverse primer)	None	None
	32	*stx1* #9a (detection probe)	CalO 560	BHQ-1
5a/6b	47	*stx2* #5F (forward primer)	None	None
	48	*stx2* #5R (reverse primer)	None	None
	49	*stx2* #5a2 (Internal Quencher BHQ-1) (detection probe)	FAM	Spacer C3
	19	*stx1* #6F (forward primer)	None	None
	20	*stx1* #6R (reverse primer)	None	None
	22	*stx1* #6b (detection probe)	CalO 560	BHQ-1

An internal control (IC) primer and probe combination was included in the stx2/stx1 mixes. The IC forward and reverse primers (designated as DNA IC 4F (Adeno) and DNA IC 4R (Adeno), respectively) had the nucleotide base sequences of SEQ ID NO:53 and SEQ ID NO:54, respectively, without 5' or 3' modification. The IC detection probe (designated as DNA TM IC 4P) had the nucleotide base sequence of SEQ ID NO:55, labeled at its 5' end with Quasar 670 and at its 3' end with BHQ-2.

Materials utilized for real-time PCR included the following: 2xFastStart PCR Master (Roche); FastStart Taq 5u/µl (Roche); AccuGENE® Molecular Biology Water; STEC genomic DNA: Escherischia coli strain EDL 933 genomic DNA, ATCC Accession Nos. 700927D and 3791888, 7.8 x 10^6 c/µl stock.

Real-time PCR reactions were initially run with different stx2 primer concentrations. stx2 forward and reverse primers were run in separate reactions at 200 nM, 300 nM, and 400 nM final concentrations for each stx2/stx1 combination. stx1 and IC forward and reverse primers were each used at a final concentration of 250 nM, stx1 and stx2 probes were each used at a final concentration of 200 nM, and IC probe was used at a final concentration of 300 nM. Final FastStart PCR Master mix was 1x, and the final concentration of FastStart Taq was 4 units. Each mix was run with the STEC genomic DNA template at 10, 1, and 0.1c/µL.

Each mix generated was frozen solid at -80° before use. For PCR, each DNA concentration and a no template control (water) were run in duplicate with a negative control in a single replicate with each mix. 20µL of the mix was added to individual Cepheid tubes and 5µL of template added and mixed via pipetting. All Cepheid tubes were spun down in a mini-centrifuge prior to PCR on the Cepheid. The cycling parameters used on the Cepheid were 95°C for 10 min (optics off), 5 cycles of 95°C for 30 sec (optics off), 55°C for 60 sec (optics on), 40 cycles of 95°C for 10 sec (optics off), 55°C for 60 sec (optics on).

The PCR runs for each mix were analyzed and based on the data, the 1a/9a and 3a/9a stx2/stx1 primer-probe combinations yielded the most consistently positive results. The Cts for the stx2 detection were very similar over the three stx2 primer concentrations tested (typically within one (1) Ct).

Further real-time PCR assays, similar to those described above, were run for the 1a/9a and 3a/9a combinations using the stx2 primers at 250 nM. The second set of assays varied the final concentration of the stx2 FAM detection probe: 200 nM, 300 nM, and 400 nM for each mix. Based on the results from these runs, subsequent assays utilized the stx2 detection probe at 250 nM for the 1a/9a combination and at 200nM for the 3a/9a combination.

A third set of assays was performed, this time varying the stx1 forward and reverse primer final concentration at 200 nM, 300 nM, and 400 nM for each mix. Based on the results of this third set, subsequent assays utilized the stx1 primers at 400 nM for the 1a/9a combination and at 250 nM for the 3a/9a combination.

A fourth set of assays was performed, this time varying the stx1 probe final concentration at 200 nM, 300 nM, and 400 nM for each mix. Based on the data from this fourth set, 200 nM was selected as a suitable concentration for the stx1 detection probe.

Concluding Statements

The contents of the articles, patents, and patent applications, and all other documents and electronically available information mentioned or cited herein, are hereby incorporated by reference in their entirety to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference. Applicants reserve the right to physically incorporate into this application any and all materials and information from any such articles, patents, patent applications, or other physical and electronic documents.

The methods illustratively described herein may be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms “comprising,” “including,” containing,” etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof. It is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the invention embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the methods. This includes the generic description of the methods with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

Other embodiments are within the following claims. In addition, where features or aspects of the methods are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

CLAIMS

1. A primer-probe set when used for identification of a *stx1* gene, said primer-probe set comprising:

a pair of amplification oligomers, each member of said pair of amplification oligomers having a length of from about 15 to about 25 contiguous nucleotides, said pair of amplification oligomers comprising

(i) a first oligomer having at least 90% sequence identity to SEQ ID NO:30 and a second oligomer having at least 90% sequence identity to SEQ ID NO:31; and

an oligomer probe hybridizable to a *stx1* gene region located between the regions of hybridization of said pair of amplification oligomers, wherein the probe comprises a detectable label.

2. The primer-probe set of claim 1, wherein said oligomer probe has a length of from about 10 to about 40 contiguous oligomer residues.

3. The primer-probe set of claim 2, wherein for the amplification oligomer pair of (i), said oligomer probe has at least 90% sequence identity to SEQ ID NO:32.

4. The primer-probe set of any one of claims 1 to 3, further comprising an internal control system for verifying reaction conditions, said system comprising a control template polynucleotide, a pair of control amplification oligomers and a control probe.

5. A kit when used for identification of a *stx1* gene, said kit comprising the primer-probe set as recited in any one of claims 1 to 4.

6. A kit when used for identification of a *stx1* gene and a *stx2* gene, said kit comprising the primer-probe set as recited in any one of claims 1 to 4 and the primer-probe set for identification of a *stx2* gene comprising:

(1) a pair of amplification oligomers, each member of said pair of amplification oligomers having a length of from about 15 to about 25 contiguous nucleotides, said pair of amplification oligomers comprising an oligomer pair selected from the group consisting of:

(i) a first oligomer having at least 90% sequence identity to SEQ ID NO:33 and a second oligomer having at least 90% sequence identity to SEQ ID NO:34;

(ii) a first oligomer having at least 90% sequence identity to SEQ ID NO:40 and a second oligomer having at least 90% sequence identity to SEQ ID NO:41;

(iii) a first oligomer having at least 90% sequence identity to SEQ ID NO:36 and a second oligomer having at least 90% sequence identity to SEQ ID NO:37; and

(iv) a first oligomer having at least 90% sequence identity to SEQ ID NO:47 and a second oligomer having at least 90% sequence identity to SEQ ID NO:48; and

(2) an oligomer probe hybridizable to a *stx2* gene region.

7. A primer-probe set when used for identification of at least one of a *stx1* gene and a *stx2* gene in a sample, said primer-probe set comprising:

a pair of *stx1*-specific amplification oligomers and a pair of *stx2*-specific amplification oligomers, each member of said *stx1*-specific and *stx2*-specific pairs of amplification oligomers having a length of from about 15 to about 25 contiguous nucleotides,

said pair of *stx1*-specific amplification oligomers comprising

(1-i) a first oligomer having at least 90% sequence identity to SEQ ID NO:30 and a second oligomer having at least 90% sequence identity to SEQ ID NO:31; and

said pair of *stx2*-specific amplification oligomers comprising an oligomer pair selected from the group consisting of

(2-i) a first oligomer having at least 90% sequence identity to SEQ ID NO:33 and a second oligomer having at least 90% sequence identity to SEQ ID NO:34;

(2-ii) a first oligomer having at least 90% sequence identity to SEQ ID NO:40 and a second oligomer having at least 90% sequence identity to SEQ ID NO:41;

(2-iii) a first oligomer having at least 90% sequence identity to SEQ ID NO:36 and a second oligomer having at least 90% sequence identity to SEQ ID NO:37; and

(2-iv) a first oligomer having at least 90% sequence identity to SEQ ID NO:47 and a second oligomer having at least 90% sequence identity to SEQ ID NO:48;

a *stx1*-specific detection probe comprising a detectable label, wherein the detection probe is hybridizable to a *stx1* gene region located between the regions of hybridization of said pair of *stx1*-specific amplification oligomers; and

a *stx2*-specific detection probe hybridizable to a *stx2* gene region located between the regions of hybridization of said pair of *stx2*-specific amplification oligomers.

8. The primer-probe set of claim 7, wherein each of said *stx1*-specific and *stx2*-specific detection probes is an oligomer having a length of from about 15 to about 30 or to about 40 contiguous oligomer residues.

9. The primer-probe set of claim 8, wherein

(I) for the amplification oligomer pair of (1-i), said *stx1*-specific probe has at least 90% sequence identity to SEQ ID NO:32; and

(II) for the amplification oligomer pair of (2-i), said *stx2*-specific probe has at least 90% sequence identity to SEQ ID NO:35;

for the amplification oligomer pair of (2-ii), said *stx2*-specific probe has at least 90% sequence identity to SEQ ID NO:42;

for the amplification oligomer pair of (2-iii), said *stx2*-specific probe has at least 90% sequence identity to SEQ ID NO:39; or

for the amplification oligomer pair of (2-iv), said *stx2*-specific probe has at least 90% sequence identity to SEQ ID NO:49.

10. The primer-probe set of any one of claims 7 to 9, wherein the *stx1*-specific and *stx2*-specific pairs of amplification oligomers are selected from the following combinations of *stx1*-specific and *stx2*-specific oligomer pairs:

(A) the amplification oligomer pairs of (1-i) and (2-i);

(B) the amplification oligomer pairs of (1-i) and (2-ii).

11. The primer-probe set of claim 10, wherein

for the combination of *stx1*-specific and *stx2*-specific oligomer pairs of (A), the *stx1*-specific probe has at least 90% sequence identity to SEQ ID NO:32 and the *stx2*-specific probe has at least 90% sequence identity to SEQ ID NO:35;

for the combination of *stx1*-specific and *stx2*-specific oligomer pairs of (B), the *stx1*-specific probe has at least 90% sequence identity to SEQ ID NO:32 and the *stx2*-specific probe has at least 90% sequence identity to SEQ ID NO:42.

12. The primer-probe set of any one of claims 7 to 11, further comprising an internal control system for verifying reaction conditions, said system comprising a control template polynucleotide, a pair of control amplification oligomers, and a control probe, optionally wherein said pair of control amplification oligomers comprises a first oligomer having at least 90%

sequence identity to SEQ ID NO:53 and a second oligomer having at least 90% sequence identity to SEQ ID NO:54, and optionally wherein said control probe is an oligomer having at least 90% sequence identity to SEQ ID NO:55.

Gen-Probe Incorporated
Patent Attorneys for the Applicant/Nominated Person
SPRUSON & FERGUSON

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<220>
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<400>  34
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<210>  35
<211>  24
<212>  DNA
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<220>
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<400>  35
ttcttcggta tcctattccc ggga                                            24


<210>  36
<211>  18
<212>  DNA
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<220>
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<400>  36
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<210>  37
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<220>
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<400>  37
ctccattaac gccagatatg                                                   20


<210>  38
<211>  26
<212>  DNA
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<220>
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<400>  38
accagtgagt gacgactgat ttgcat                                            26


<210>  39
<211>  23
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<220>
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<400>  39
ataccactct gcaacgtgtc gca                                               23


<210>  40
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<400>  40
cagagatgca tccagagc                                                     18


<210>  41
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<212>  DNA
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<220>
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<400>  41
ccggaagcac attgctga                                                     18


<210>  42
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<220>
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<400>  42
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<210>  43
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<220>
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<400>  43
acaggagcag tttcagacag tgc                                               23


<210>  44
<211>  18
<212>  DNA
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<220>
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<400>  44
ggacctcact ctgaactg                                                     18


<210>  45
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<400>  45
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<210>  46
<211>  24
<212>  DNA
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<220>
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<400>  46
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<210>  47
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<220>
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<400>  47
gaatgtcaga taagtggcga                                                   20


<210>  48
<211>  20
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<220>
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<400>  48
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<210>  49
<211>  29
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<220>
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<400>  49
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<210>  50
<211>  21
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<220>
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<400>  50
caggagcgta ggaataccat c                                                 21


<210>  51
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<400>  51
gggctaactg tgaagattca atag                                              24


<210>  52
<211>  28
<212>  DNA
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<220>
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<400>  52
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tcactgattc aagcacgtta gaaggcca 28

<210> 53
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<220>
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<400> 53
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<210> 54
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<212> DNA
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<220>
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<400> 54
gacttgtggc cttctaacg 19

<210> 55
<211> 25
<212> DNA
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<220>
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<400> 55
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<210> 56
<211> 1230
<212> DNA
<213> Escherichia coli

<300>
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<309> 2008-01-18
<313> (1)..(1230)

<400> 56
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gtggttgcga aggaatttac cttagacttc tcgactgcaa agacgtatgt agattcgctg 120

aatgtcattc gctctgcaat aggtactcca ttacagacta tttcatcagg aggtacgtct 180

ttactgatga ttgatagtgg cacaggggat aatttgtttg cagttgatgt cagagggata 240

gatccagagg aagggcggtt taataatcta cggcttattg ttgaacgaaa taatttatat 300

gtgacaggat ttgttaacag gacaaataat gtttttatc gctttgctga tttttcacat 360

gttacctttc caggtacaac agcggttaca ttgtctggtg acagtagcta taccacgtta 420 cagcgtgttg cagggatcag tcgtacgggg atgcagataa atcgccattc gttgactact 480

tcttatctgg atttaatgtc gcatagtgga acctcactga cgcagtctgt ggcaagagcg 540

atgttacggt ttgttactgt gacagctgaa gctttacgtt ttcggcaaat acagagggga 600

tttcgtacaa cactggatga tctcagtggg cgttcttatg taatgactgc tgaagatgtt 660

gatcttacat tgaactgggg aaggttgagt agtgtcctgc ctgattatca tggacaagac 720

tctgttcgtg taggaagaat ttcttttgga agcattaatg caattctggg aagcgtggca 780

ttaatactga attgtcatca tcatgcatcg cgagttgcca gaatggcatc tgatgagttt 840

ccttctatgt gtccggcaga tggaagagtc cgtgggatta cgcacaataa aatattgtgg 900

gattcatcca ctctgggggc aattctgatg cgcagaacta ttagcagttg agggggtaaa 960

atgaaaaaaa cattattaat agctgcatcg ctttcatttt tttcagcaag tgcgctggcg 1020

acgcctgatt gtgtaactgg aaaggtggag tatacaaaat ataatgatga cgataccttt 1080

acagttaaag tgggtgataa agaattattt accaacagat ggaatcttca gtctcttctt 1140

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gggggattca gcgaagttat ttttcgttga 1230

<210> 57
<211> 1241
<212> DNA
<213> Escherichia coli

<300>
<308> GB Acc. No. BA000007; gi:47118301; bases 1266965 to 1268205
<309> 2008-01-18
<313> (1)..(1241)

<400> 57
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agtatacgga cagagatatc gacccctctt gaacatatat ctcaggggac cacatcggtg 180

tctgttatta accacacccc accgggcagt tattttgctg tggatatacg agggcttgat 240

gtctatcagg cgcgttttga ccatcttcgt ctgattattg agcaaaataa tttatatgtg 300

gccgggttcg ttaatacggc aacaaatact ttctaccgtt tttcagattt tacacatata 360

tcagtgcccg gtgtgacaac ggtttccatg acaacggaca gcagttatac cactctgcaa 420

cgtgtcgcag cgctggaacg ttccggaatg caaatcagtc gtcactcact ggtttcatca 480

tatctggcgt taatggagtt cagtggtaat acaatgacca gagatgcatc cagagcagtt 540

ctgcgttttg tcactgtcac agcagaagcc ttacgcttca ggcagataca gagagaattt 600

cgtcaggcac tgtctgaaac tgctcctgtg tatacgatga cgccgggaga cgtggacctc 660

actctgaact gggggcgaat cagcaatgtg cttccggagt atcggggaga ggatggtgtc 720

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gaatgtcaga taactggcga caggcctgtt ataaaaataa acaatacatt atgggaaagt      900
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caatggcggc ggattgtgct aaaggtaaaa ttgagttttc caagtataat gaggatgaca     1080
catttacagt gaaggttgac gggaaagaat actggaccag tcgctggaat ctgcaaccgt     1140
tactgcaaag tgctcagttg acaggaatga ctgtcacaat caaatccagt acctgtgaat     1200
caggctccgg atttgctgaa gtgcagttta ataatgactg a                          1241
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