Abstract:

Title: SEQUENCES AND THEIR USE FOR DETECTION OF SALMONELLA

This invention relates to a rapid method for detection of Salmonella in a sample based on the presence of nucleic acid sequences, in particular, to a PCR-based method for detection, and to oligonucleotide molecules and reagents and kits useful therefor. In certain embodiments, the method is employed to detect Salmonella in a food or water sample. The present invention further relates to isolated polynucleotides, replication compositions, and kits for carrying out the method of the present invention.
This invention relates to a rapid method for detection of Salmonella in a sample based on the presence of nucleic acid sequences, in particular, to a PCR-based method for detection, and to oligonucleotide molecules and reagents and kits useful therefore. In certain embodiments, the method is employed to detect Salmonella in a food or water sample. The present invention further relates to isolated polynucleotides, replication compositions, and kits for carrying out the method of the present invention.

Salmonella is a genus of rod-shaped, gram-negative bacteria that is known to cause numerous illnesses, including food poisoning and typhoid fever. Salmonella infections can be transferred from animals to humans and can be acquired through the ingestion of food contaminated with Salmonella. In infections involving entirid type Salmonella, which is responsible for food poisoning, the organism typically enters the digestive tract through ingestion. In healthy adults, Salmonella generally must be ingested in large numbers to cause any disease. However, in young children, ingestion of a relatively small number of bacteria has been shown to cause disease due to the increased susceptibility of this population. With regard to the course of Salmonella infection, the bacteria typically incubate for up to one day before symptoms of infection appear. After that incubation period, intestinal inflammation occurs, resulting in diarrhea that is often bloody. Symptoms are generally mild with no sepsis, though sepsis can occur in immunocompromised individuals. Additionally, Salmonella meningitis can occur in children.

Because of its mode of transmission and the seriousness of some infections, detection of Salmonella in samples, such as food or beverage samples, is critical to
the safety of the population. As such, it is desirable to have a test for the rapid and accurate detection of *Salmonella* in a sample.

**SUMMARY OF INVENTION**

One aspect of this invention is a method for detecting the presence of *Salmonella* in a sample, the sample comprising nucleic acids, the method comprising (a) providing a reaction mixture comprising at least one primer and probe, wherein the primer is at least 11 nucleotides in length and the probe is at least 14 nucleotides in length, and wherein (i) the primer comprises a nucleic acid sequence capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO: 1 or a sequence complementary thereto, and the probe comprises a nucleic acid sequence capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO: 2 or a sequence complementary thereto; or (ii) the primer comprises a nucleic acid sequence capable of selectively hybridizing under stringent conditions to the nucleic acid sequence of SEQ ID NO: 3 or a sequence complementary thereto, and the probe comprises a nucleic acid sequence capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO: 4 or a sequence complementary thereto; and (b) performing PCR amplification of the nucleic acids of the sample using the reaction mixture of step (a); and (c) detecting the amplification of step (b).

In certain embodiments, the reaction mixture comprises both the primer and probe of (i) and the primer and probe of (ii). In certain other embodiments the primer and probe each possess a 3' terminus and a 5' terminus, and the 3' terminus of said probe is directly or indirectly attached to the 5' terminus of the primer, thereby forming a primer-probe complex. In still further embodiments, the primer-probe complex further comprises a 5' Stem Sequence and a 3' Stem Sequence, wherein the 3' terminus of the 5' Stem Sequence is directly or indirectly attached to the 5' terminus of the probe, wherein the 5' terminus of the 3' Stem Sequence is directly or indirectly attached to the 3' terminus of the probe, and wherein the 3' terminus of the 3' Stem Sequence is directly or indirectly attached to the 5' terminus of the primer. In additional embodiments, the primer-probe complex comprises a detectable label. In
further embodiments, the reaction mixture further comprises a quencher oligonucleotide capable of selectively hybridizing under stringent conditions to the probe.

In certain examples, the primer capable of selectively hybridizing to SEQ ID NO: 1 comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 40-54, and the probe capable of selectively hybridizing to SEQ ID NO: 2 comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 6-39. In other examples, the primer capable of selectively hybridizing to SEQ ID NO: 3 comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 95-111, and wherein said probe capable of selectively hybridizing to SEQ ID NO: 4 comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 55-94. In further examples, the 5' Stem Sequence comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 112-119, and wherein said 3' Stem Sequence comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 120-130.

In another aspect, the invention relates to an isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NOs: 6-111. In other embodiments, the invention relates to an isolated polynucleotide comprising a primer-probe complex, wherein the primer-probe complex comprises a nucleic acid primer portion and a nucleic acid probe portion, wherein the primer portion is at least 11 nucleotides in length and the probe portion is at least 14 nucleotides in length, wherein the 3' terminus of the probe portion is directly or indirectly attached to the 5' terminus of the primer portion, and wherein (i) the primer portion comprises a nucleic acid sequence capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO: 1 or a sequence complementary thereto, and the probe portion comprises a nucleic acid sequence capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO: 2 or a sequence complementary thereto; or (ii) the primer portion comprises a nucleic acid sequence capable of selectively hybridizing under stringent conditions to the nucleic acid sequence of SEQ ID NO: 3 or a sequence complementary thereto, and the probe portion comprises a nucleic acid
sequence capable of selectively hybridizing under stringent conditions to the
sequence of SEQ ID NO: 4 or a sequence complementary thereto. In certain
examples, the primer portion comprises a nucleic acid sequence selected from the
group consisting of SEQ ID NOs: 40-54, and/or the probe portion comprises a nucleic
acid sequence selected from the group consisting of SEQ ID NOs: 6-39. In other
examples, the primer portion comprises a nucleic acid sequence selected from the
group consisting of SEQ ID NOs: 95-111, and/or the probe portion comprises a
nucleic acid sequence selected from the group consisting of SEQ ID NOs: 55-94.

In additional embodiments, the primer-probe complex further comprises a 5'
Stem Sequence and a 3' Stem Sequence, wherein the 3' terminus of the 5' Stem
Sequence is directly or indirectly attached to the 5' terminus of the probe portion,
wherein the 5' terminus of the 3' Stem Sequence is directly or indirectly attached to
the 3' terminus of the probe portion, and wherein the 3' terminus of the 3' Stem
Sequence is directly or indirectly attached of the 5' terminus of the primer portion. In
certain examples, the 5' Stem Sequence comprises a nucleic acid sequence selected
from the group consisting of SEQ ID NOs: 112-119, and/or the 3' Stem Sequence
comprises a nucleic acid sequence selected from the group consisting of SEQ ID
NOs: 120-130.

In other aspects, the invention relates to a reagent tablet or kit for detection of
Salmonella in a sample.

SUMMARY OF THE SEQUENCES
SEQ ID NOs: 1-5 are the nucleotide sequences of portions of the Salmonella
genome that are useful for detecting the presence of Salmonella in a sample. In
certain examples, a primer directed toward SEQ ID NO: 1 is used in conjunction with
a probe directed toward SEQ ID NO: 2. In other examples, a primer directed toward
SEQ ID NO: 3 is used in conjunction with a probe directed toward SEQ ID NO: 4 or
5. In certain other examples, the primer and probe are attached so as to form a
primer-probe complex, wherein the 3' terminus of the probe portion is directly or
indirectly attached to the 5' terminus of the primer portion. Primers and/or probes
capable of selectively hybridizing under stringent conditions to SEQ ID NO: 1 include
SEQ ID NOs: 40-54. Primers and/or probes capable of selectively hybridizing under stringent conditions to SEQ ID NO: 2 include SEQ ID NOs: 6-39. Primers and/or probes capable of selectively hybridizing under stringent conditions to SEQ ID NO: 3 include SEQ ID NOs: 95-1 11. Primers and/or probes capable of selectively hybridizing under stringent conditions to SEQ ID NO: 4 include SEQ ID NOs: 55-94.

SEQ ID NOs: 6-39 are nucleotide sequences capable of use as primers or probes for selectively hybridizing under stringent conditions, and ultimately detecting, the sequence of SEQ ID NO: 2.

SEQ ID NOs: 40-54 are nucleotide sequences capable of use as primers or probes for selectively hybridizing under stringent conditions, and ultimately detecting, the sequence of SEQ ID NO: 1.

SEQ ID NOs: 55-94 are nucleotide sequences capable of use as primers or probes for selectively hybridizing under stringent conditions, and ultimately detecting, the sequence of SEQ ID NO: 4 or .

SEQ ID NOs: 95-1 11 are nucleotide sequences capable of use as primers or probes for selectively hybridizing under stringent conditions, and ultimately detecting, the sequence of SEQ ID NO: 3.

SEQ ID NOs: 112-1 19 are nucleotide sequences capable of use as a 5' Stem Sequence, for example, in conjunction with a suitable probe sequence, such as those described by SEQ ID NOs: 6-39 and 55-94. In certain examples, the 5' Stem Sequence is directly or indirectly attached to a probe sequence, such that the 3' terminus of the 5' Stem Sequence is directly or indirectly attached to the 5' terminus of the probe sequence.

SEQ ID NOs: 120-1 30 are nucleotide sequences capable of use as a 3' Stem Sequence, for example, in conjunction with a suitable probe sequence, such as those described by SEQ ID NOs: 6-39 and 55-94. In certain examples, the 3' Stem Sequence is directly or indirectly attached to a probe sequence and a primer sequence, such that the 5' terminus of the 3' Stem Sequence is directly or indirectly attached to the 3' terminus of the probe sequence and the 3' terminus of the 3' Stem Sequence is directly or indirectly attached to the 5' terminus of the primer sequence.
SEQ ID NO: 131 is the nucleotide sequence that comprises a synthetic SV40 ("sSV40") sequence which can be effectively employed, for example, as a target for a positive control amplification reaction. In certain embodiments, this sequence can be used as a "spiked" control and can be amplified and detected using SEQ ID NOs: 132-136.

SEQ ID NOs: 132-136 are nucleotide sequences useful for amplifying and detecting the Positive Control sequence of SEQ ID NO: 131. In certain embodiments, SEQ ID NO: 132 is a 5' stem sequence, SEQ ID NO: 133 is a probe sequence, SEQ ID NO: 134 is a 3' stem sequence, SEQ ID NO 135 is a forward primer sequence, and SEQ ID NO: 136 is a reverse primer sequence. In other embodiments, SEQ ID NOs: 132-135 are combined so as to form a primer-probe complex capable of forming a stem-loop structure. In other embodiments that primer-probe complex is used as a forward primer/probe in conjunction with SEQ ID NO: 136 as reverse primer to amplify and detect SEQ ID NO: 131.

DETAILS DESCRIPTION

Applicants specifically incorporate the entire contents of all cited references in this disclosure. Further, when an amount, concentration, or other value or parameter is given as either a range, preferred range, or a list of upper preferable values and lower preferable values, this is to be understood as specifically disclosing all ranges formed from any pair of any upper range limit or preferred value and any lower range limit or preferred value, regardless of whether ranges are separately disclosed. Where a range of numerical values is recited herein, unless otherwise stated, the range is intended to include the endpoints thereof, and all integers and fractions within the range. It is not intended that the scope of the invention be limited to the specific values recited when defining a range.

Definitions

In this disclosure, a number of terms and abbreviations are used. The following definitions are provided.
As used herein, the term "about" or "approximately" means within 20%, preferably within 10%, and more preferably within 5% of a given value or range.

The term "comprising" is intended to include embodiments encompassed by the terms "consisting essentially of" and "consisting of." Similarly, the term "consisting essentially of" is intended to include embodiments encompassed by the term "consisting of."

"Polymerase chain reaction" is abbreviated PCR.

The term "isolated" refers to materials, such as nucleic acid molecules and/or proteins, which are substantially free or otherwise removed from components that normally accompany or interact with the materials in a naturally occurring environment. Isolated polynucleotides may be purified from a host cell in which they naturally occur. Conventional nucleic acid purification methods known to skilled artisans may be used to obtain isolated polynucleotides. The term also embraces recombinant polynucleotides and chemically synthesized polynucleotides.

The terms "polynucleotide," "polynucleotide sequence," "nucleic acid sequence," "nucleic acid fragment," and "oligonucleotide" are used interchangeably herein. These terms encompass nucleotide sequences and the like. A polynucleotide may be a polymer of RNA or DNA that is single- or double-stranded, that optionally contains synthetic, non-natural, or altered nucleotide bases. A polynucleotide in the form of a polymer of DNA may be comprised of one or more strands of cDNA, genomic DNA, synthetic DNA, or mixtures thereof.

The term "amplification product" or "amplicon" refers to nucleic acid fragments produced during a primer-directed amplification reaction. Typical methods of primer-directed amplification include polymerase chain reaction (PCR), ligase chain reaction (LCR), or strand displacement amplification (SDA). If PCR methodology is selected, the replication composition may comprise the components for nucleic acid replication, for example: nucleotide triphosphates, two (or more) primers or primer-probe complexes with appropriate sequences, thermostable polymerase, buffers, solutes, and proteins. These reagents and details describing procedures for their use in amplifying nucleic acids are provided in U.S. Patent No. 4,683,202 (1987, Mullis, et al.) and U.S. Patent No. 4,683,195 (1986, Mullis, et al.). If LCR methodology is
selected, then the nucleic acid replication compositions may comprise, for example: a thermostable ligase (e.g., *Thermus aquaticus* ligase), two sets of adjacent oligonucleotides (wherein one member of each set is complementary to each of the target strands), Tris-HCl buffer, KCl, EDTA, NAD, dithiothreitol, and salmon sperm DNA. See, for example, Tabor et al., *Proc. Natl. Acad. Sci. U.S.A.* 82:1 074-1 078 (1985).

The term "primer" refers to an oligonucleotide (synthetic or occurring naturally) that is capable of acting as a point of initiation of nucleic acid synthesis or replication along a complementary strand when placed under conditions in which synthesis of a complementary strand is catalyzed by a polymerase. A primer can further contain a detectable label, for example a 5' end label. In certain embodiments, primers of the present invention are 8-60 nucleic acids in length. In other embodiments, primers are 10-50, 14-40, or 20-30 nucleic acids in length. In certain specific embodiments, primers are at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length.

The term "probe" refers to an oligonucleotide (synthetic or occurring naturally) that is complementary (though not necessarily fully complementary) to a polynucleotide of interest and forms a duplexed structure by hybridization with at least one strand of the polynucleotide of interest. A probe or primer-probe complex can further contain a detectable label. In certain embodiments, probes of the present invention are 8-60 nucleic acids in length. In other embodiments, probes are 10-50, 14-40, or 20-30 nucleic acids in length. In certain specific embodiments, probes are at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length.

A probe can either be an independent entity or complexed with or otherwise attached to a primer, such as where a probe is connected, directly or indirectly, via its 3' terminus to a primer's 5' terminus. In some examples, the probe and primer are attached through a linker, which may be a nucleotide or non-nucleotide linker and which may be a non-amplifiable linker, such as a hexethylene glycol (HEG) or 18-carbon linker. In such a case, this would be termed a "primer-probe complex." One example of such primer-probe complexes can be found in U.S. Patent No. 6,326,145,
incorporated herein by reference in its entirety, which are frequently referred to as
"Scorpion probes" or "Scorpion primers." In a typical primer probe complex, the
primer portion can be, for example, at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20,
21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length, while the probe portion
can be, for example, at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24,
25, 26, 27, 28, 29, or 30 nucleotides in length.

As used herein, the terms "label" and "detectable label" refer to a molecule
capable of detection, including, but not limited to, radioactive isotopes, fluorescers,
chemiluminescers, enzymes, enzyme substrates, enzyme cofactors, enzyme
inhibitors, chromophores, dyes, metal ions, metal sols, semiconductor nanocrystals,
ligands (e.g., biotin, avidin, streptavidin, or haptens), and the like. A detectable label
can also include a combination of a reporter and a quencher.

The term "reporter" refers to a substance or a portion thereof that is capable of
exhibiting a detectable signal, which signal can be suppressed by a quencher. The
detectable signal of the reporter is, e.g., fluorescence in the detectable range. The
term "quencher" refers to a substance or portion thereof that is capable of
suppressing, reducing, inhibiting, etc., the detectable signal produced by the reporter.

As used herein, the terms "quenching" and "fluorescence energy transfer"
refer to the process whereby, when a reporter and a quencher are in close proximity,
and the reporter is excited by an energy source, a substantial portion of the energy of
the excited state nonradiatively transfers to the quencher where it either dissipates
nonradiatively or is emitted at a different emission wavelength than that of the
reporter.

Preferably, the reporter may be selected from fluorescent organic dyes
modified with a suitable linking group for attachment to the oligonucleotide, such as
to the terminal 3' carbon or terminal 5' carbon. The quencher may also be selected
from organic dyes, which may or may not be fluorescent, depending on the
embodiment of the present invention. Generally, whether the quencher is fluorescent
or simply releases the transferred energy from the reporter by non-radiative decay,
the absorption band of the quencher should at least substantially overlap the
fluorescent emission band of the reporter to optimize the quenching. Non-fluorescent
quenchers or dark quenchers typically function by absorbing energy from excited reporters, but do not release the energy radiatively.

Selection of appropriate reporter-quencher pairs for particular probes may be undertaken in accordance with known techniques. Fluorescent and dark quenchers and their relevant optical properties from which exemplary reporter-quencher pairs may be selected are listed and described, for example, in Berlman, Handbook of Fluorescence Spectra of Aromatic Molecules, 2nd ed., Academic Press, New York, 1971, the content of which is incorporated herein by reference. Examples of modifying reporters and quenchers for covalent attachment via common reactive groups that can be added to an oligonucleotide in the present invention may be found, for example, in Haugland, Handbook of Fluorescent Probes and Research Chemicals, Molecular Probes of Eugene, Oreg., 1992, the content of which is incorporated herein by reference.

Preferred reporter-quencher pairs may be selected from xanthene dyes including fluoresceins and rhodamine dyes. Many suitable forms of these compounds are available commercially with substituents on the phenyl groups, which can be used as the site for bonding or as the bonding functionality for attachment to an oligonucleotide. Another preferred group of fluorescent compounds for use as reporters is the naphthylamines, having an amino group in the alpha or beta position.

Included among such naphthylamino compounds are 1-dimethylaminonaphthyl-5-sulfonate, 1-anilino-8-naphthalene sulfonate and 2-p-touidinyl-6-naphthalene sulfonate. Other dyes include 3-phenyl-7-isocyanatocoumarin; acridines such as 9-isothiocyanatoacridine; N-(p-(2-benzoxazolyl)phenyl)maleimide; benzoxadiazoles; stilbenes; pyrenes and the like.

Most preferably, the reporters and quenchers are selected from fluorescein and rhodamine dyes. These dyes and appropriate linking methodologies for attachment to oligonucleotides are well known in the art.

Suitable examples of quenchers may be selected from 6-carboxy-tetramethyl-rhodamine, 4-(4-dimethylaminophenylazo) benzoic acid (DABYL), tetramethylrhodamine (TAMRA), BHQ-0™, BHQ-1™, BHQ-2™, and BHQ-3™, each of which are available from Biosearch Technologies, Inc. of Novato, Calif., QSY-7™,
QSY-9™, QSY-21™ and QSY-35™, each of which are available from Molecular Probes, Inc., and the like.

Suitable examples of reporters may be selected from dyes such as SYBR green, 5-carboxyfluorescein (5-FAM™ available from Applied Biosystems of Foster City, Calif.), 6-carboxyfluorescein (6-FAM), tetrachloro-6-carboxyfluorescein (TET), 2,7-dimethoxy-4,5-dichloro-6-carboxyfluorescein, hexachloro-6-carboxyfluorescein (HEX), 6-carboxy-2',4,7,7'-tetrachlorofluorescein (6-TET™ available from Applied Biosystems), carboxy-X-rhodamine (ROX), 6-carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein (6-JOE™ available from Applied Biosystems), VIC™ dye products available from Molecular Probes, Inc., NED™ dye products available from Applied Biosystems, and the like.

One example of a probe that contains a reporter and a quencher is a Scorpion probe in either a unimolecular or bimolecular conformation. In a unimolecular Scorpion, the probe portion of the primer-probe complex is flanked by self-complementary regions, which allow the probe to form into a stem-loop structure when the probe is unbound from its target DNA. In certain embodiments, these are termed the 5' Stem Sequence, which has its 3' terminus attached to the 5' terminus of the probe, and the 3' Stem Sequence, which has its 5' terminus attached to the 3' terminus of the probe and its 3' terminus attached to the primer. These attachments can be either direct or indirect, such as through a linker. Further, in a unimolecular Scorpion, a reporter is typically attached at or near one of the self-complementary regions, such as at the 5' terminus of the Scorpion probe, and a quencher is attached at or near the other self-complementary region, such as immediately 5' to the non-amplifiable linker, such that the quencher is in sufficiently close proximity to the reporter to cause quenching when the probe is in its stem-loop conformation. In a bimolecular Scorpion, self-complementary flanking regions are not typically employed, but rather a separate "blocking oligonucleotide" or "quenching oligonucleotide" is employed in conjunction with the Scorpion probe. This blocking oligonucleotide is capable of hybridizing to the probe region of the Scorpion probe when the probe is unbound from its target DNA. Further, in a bimolecular Scorpion, the reporter is typically attached to the probe region of the Scorpion probe, such as at
the 5' terminus of the Scorpion probe, while the quencher is attached to the blocking oligonucleotide, such as at the 3' terminus of the blocking oligonucleotide, such that the quencher is in sufficiently close proximity to the reporter to cause quenching when the probe is unbound from its target DNA and is instead hybridized to the blocking oligonucleotide.

Another example of a probe that contains a reporter and a quencher is a probe that is to be used in a 5'-exonuclease assay, such as the TaqMan® real-time PCR technique. In this context, the oligonucleotide probe will have a sufficient number of phosphodiester linkages adjacent to its 5' end so that the 5' to 3' nuclease activity employed can efficiently degrade the bound probe to separate the reporters and quenchers. Yet another example of a probe that contains a reporter and quencher is a Molecular Beacon type probe, which contains a probe region flanked by self-complementary regions that allow the probe to form a stem-loop structure when unbound from the probe's target sequence. Such probes typically have a reporter attached at or near one terminus and a quencher attached at or near the other terminus such that the quencher is in sufficiently close proximity to the reporter to cause quenching when the probe is in its unbound, and thus stem-loop, form.

The term "replication inhibitor moiety" refers to any atom, molecule or chemical group that is attached to the 3' terminal hydroxyl group of an oligonucleotide that will block the initiation of chain extension for replication of a nucleic acid strand. Examples include, but are not limited to: 3'-deoxynucleotides (e.g., cordycepin), dideoxynucleotides, phosphate, ligands (e.g., biotin and dinitrophenol), reporter molecules (e.g., fluorescein and rhodamine), carbon chains (e.g., propanol), a mismatched nucleotide or polynucleotide, or peptide nucleic acid units. The term "non-participatory" refers to the lack of participation of a probe or primer in a reaction for the amplification of a nucleic acid molecule. Specifically a non-participatory probe or primer is one that will not serve as a substrate for, or be extended by, a DNA or RNA polymerase. A "non-participatory probe" is inherently incapable of being chain extended by a polymerase. It may or may not have a replication inhibitor moiety.

A nucleic acid molecule is "hybridizable" to another nucleic acid molecule, such as a cDNA, genomic DNA, or RNA, when a single stranded form of the nucleic
acid molecule can anneal to the other nucleic acid molecule under the appropriate conditions of temperature and solution ionic strength. Hybridization and washing conditions are well known and exemplified, for example, in Sambrook, J., Fritsch, E. F. and Maniatis, T., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory: Cold Spring Harbor, NY (1989), particularly Chapter 11 and Table 11.1 therein (entirely incorporated herein by reference). The conditions of temperature and ionic strength determine the "stringency" of the hybridization. For preliminary screening for homologous nucleic acids, low stringency hybridization conditions, corresponding to a Tm of 55°C, can be used, e.g., 5X SSC, 0.1% SDS, 0.25% milk, and no formamide; or 30% formamide, 5X SSC, 0.5% SDS. Moderate stringency hybridization conditions correspond to a higher Tm, e.g., 40% formamide, with 5X or 6X SSC. Hybridization requires that the two nucleic acids contain complementary sequences, although, depending on the stringency of the hybridization, mismatches between bases are possible. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementation, variables well known in the art. The greater the degree of similarity or homology between two nucleotide sequences, the greater the value of Tm for hybrids of nucleic acids having those sequences. The relative stability (corresponding to higher Tm) of nucleic acid hybridizations decreases in the following order: RNA:RNA, DNA:RNA, DNA:DNA. For hybrids of greater than 100 nucleotides in length, equations for calculating Tm have been derived (see Sambrook et al., *supra*, 9.50-9.51). For hybridizations with shorter nucleic acids, i.e., oligonucleotides, the position of mismatches becomes more important, and the length of the oligonucleotide determines its specificity (see Sambrook et al., *supra*, 11.7-11.8). In one preferred embodiment, the length for a hybridizable nucleic acid is at least about 10 nucleotides. More preferably a minimum length for a hybridizable nucleic acid is at least about 11 nucleotides, at least about 12 nucleotides, at least about 13 nucleotides, at least about 14 nucleotides, at least about 15 nucleotides, at least about 16 nucleotides, at least about 17 nucleotides, at least about 18 nucleotides, at least about 19 nucleotides, at least about 20 nucleotides, at least about 21 nucleotides, at least about 22 nucleotides, at least about 23 nucleotides, at least
about 24 nucleotides, at least about 25 nucleotides, at least about 26 nucleotides, at least about 27 nucleotides, at least about 28 nucleotides, at least about 29 nucleotides, or, most preferably, at least 30 nucleotides. Furthermore, the skilled artisan will recognize that the temperature and wash solution salt concentration may be adjusted as necessary according to factors such as length of the probe.

In certain embodiments, primers probes are able to selectively hybridize to a target nucleic acid sequence under selective (e.g., stringent) hybridization conditions. The term "selectively hybridize" includes reference to hybridization, under stringent hybridization conditions, of a nucleic acid sequence to a specified nucleic acid target sequence to a detectably greater degree (e.g., at least 2-fold over background) than its hybridization to non-target nucleic acid sequences and to the substantial exclusion of non-target nucleic acids. Selectively hybridizing nucleic acid sequences typically have about at least 70% sequence identity, preferably at least 80% sequence identity, and most preferably 90%, 95%, 97%, 99%, or 100% sequence identity with each other.

Standard recombinant DNA and molecular cloning techniques used here are well known in the art and are described by, e.g., Sambrook et al. (supra); and by Ausubel, F. M. et al., Current Protocols in Molecular Biology, published by Greene Publishing Assoc. and Wiley-Interscience (1987).

Oligonucleotides

Methods have been developed for detecting Salmonella bacteria in a sample by detecting one or more target nucleic acid sequences. In certain embodiments, the methods involve isolated polynucleotides and/or reaction mixtures comprising a primer and a probe, wherein (i) the primer is capable of selectively hybridizing under stringent conditions to SEQ ID NO: 1 or a sequence complementary thereto and the probe is capable of selectively hybridizing under stringent conditions to SEQ ID NO: 2 or a sequence complementary thereto; or (ii) the primer is capable of selectively hybridizing under stringent conditions to SEQ ID NO: 3 or a sequence complementary thereto and the probe is capable of selectively hybridizing under stringent conditions to SEQ ID NO: 4 or a sequence complementary thereto. In some
embodiments, probes capable of selectively hybridize to SEQ ID NO: 1 include SEQ ID NOs: 40-54. In other embodiments, primers capable of selectively hybridize to SEQ ID NO: 2 include SEQ ID NOs: 6-39. In further embodiments, probes capable of selectively hybridize to SEQ ID NO: 3 include SEQ ID NOs: 95-111. In still further embodiments, primers capable of selectively hybridize to SEQ ID NO: 4 include SEQ ID NOs: 55-94.

In some embodiments, the primer and probe are directly or indirectly attached, thereby forming a primer-probe complex. In some examples, the primer-probed complex is formed by directly or indirectly attaching 3' terminus of the probe to the 5' terminus of the primer. These primer probe complexes of the instant invention can also contain a non-amplifiable linker that connects the 3' terminus of the probe region to the 5' terminus of the primer region. This non-amplifiable linker stops extension of a complementary strand from proceeding into the probe region of the primer-probe complex. Examples of such non-amplifiable linkages include hexethylene glycol (HEG) and, preferably, 18-carbon linkers.

Primer-probe complexes of the present invention can also contain a self-complementary region, including a 3' Stem Sequence and a 5' Stem Sequence, that allows the primer-probe complex to form a stem-loop structure when the probe is unbound from its target DNA, which may be useful, for example, in bringing the reporter and quencher into sufficiently close proximity to one another to cause the reporter signal to be quenched. In some embodiments, the 5' Stem Sequence is one of SEQ ID NOs: 112-119, and the 3' Stem Sequence is one of SEQ ID NOs: 120-130.

In additional embodiments, the primers, probes, or primer-probe complexes further comprise a detectable label, such as a 5' end label or a reporter-quencher pair. In some instances, a quencher oligonucleotide can be employed with a probe or primer-probe complex, which quencher oligonucleotide is capable of hybridizing to the probe or probe region of the primer-probe complex when the probe is unbound from its target DNA. If the reporter is attached to the probe or primer-probe complex and the quencher is attached to the blocking oligonucleotide, this can bring the
reporter and quencher into sufficiently close proximity to one another to allow quenching to occur.

In certain embodiments, the primer or primer-probe complex is used in conjunction with a reverse primer. In still further embodiments, two such primer-probe complexes are employed, one as a forward primer-probe complex and the other as a reverse primer-probe complex. Exemplary combinations of probe portions, primer portions, 5′ and 3′ Stem Sequences, linking moieties, and detectable labels are provided in Figs. 1A-1C.

In addition to their usefulness in PCR, these primer-probe complexes may also be useful for other nucleic acid amplification methods such as the ligase chain reaction (LCR) (Backman et al., 1989, EP 0 320 308; Carrino et al., 1995, J. Microbiol. Methods 23: 3-20); nucleic acid sequence-based amplification (NASBA), (Carrino et al., 1995, supra); and self-sustained sequence replication (3SR) and ‘Q replicase amplification’ (Pfeffer et al., 1995 Veterinary Res. Comm. 19:375-407).

In addition, oligonucleotides of the present invention also may be used as hybridization probes. Hybridization using DNA probes has been frequently used for the detection of pathogens in food, clinical and environmental samples, and the methodologies are generally known to one skilled in the art. It is generally recognized that the degree of sensitivity and specificity of probe hybridization is lower than that achieved through the previously described amplification techniques.

Assay Methods

Detection of the selected gene targets, and subsequent detection of the presence of Salmonella in a sample, may be accomplished in any suitable manner. Preferred methods are primer-directed amplification methods and nucleic acid hybridization methods. These methods may be used to detect Salmonella in a sample that is either a complex matrix or a purified culture, e.g., from an animal, environmental, or food source suspected of contamination.

A preferred embodiment of the instant invention comprises (1) culturing a complex sample mixture in a non-selective growth media to resuscitate the target bacteria, (2) releasing total target bacterial DNA, and (3) subjecting the total DNA to
an amplification protocol with a primer and probe, or a primer-probe complex, of the invention and a reverse primer, or two primer-probe complexes of the invention (one acting as a forward primer and a second acting as a reverse primer.

**Primer-Directed Amplification Assay Methods**

A variety of primer-directed nucleic acid amplification methods are known in the art which can be employed in the present invention, including thermal cycling methods (e.g., PCR, RT-PCR, and LCR), as well as isothermal methods and strand displacement amplification (SDA). The preferred method is PCR. In one preferred embodiment, the primer-probe complexes set forth in Figs. 1A-1C may be used as primers for use in primer-directed nucleic acid amplification for the detection of the target nucleic acid(s) and, ultimately, the detection of *Salmonella*.

**Sample Preparation:**

The oligonucleotides and methods according to the instant invention may be used directly with any suitable clinical or environmental samples, without any need for sample preparation. In order to achieve higher sensitivity, and in situations where time is not a limiting factor, it is preferred that the samples be pre-treated and that pre-amplification enrichment is performed.

The minimum industry standard for the detection of food-borne bacterial pathogens is a method that will reliably detect the presence of one pathogen cell in 25 g of food matrix as described in Andrews et al., 1984, "Food Sample and Preparation of Sample Homogenate", Chapter 1 in *Bacteriological Analytical Manual*, 8th Edition, Revision A, Association of Official Analytical Chemists, Arlington, VA. In order to satisfy this stringent criterion, enrichment methods and media have been developed to enhance the growth of the target pathogen cell in order to facilitate its detection by biochemical, immunological or nucleic acid hybridization means. Typical enrichment procedures employ media that will enhance the growth and health of the target bacteria and also inhibit the growth of any background or non-target microorganisms present. For example, the USDA has set forth a protocol for
enrichment of samples of ground beef to be tested for pathogenic *E. coli* (U.S. Food and Drug Administration, Bacterial Analytical Manual).

Selective media have been developed for a variety of bacterial pathogens and one of skill in the art will know to select a medium appropriate for the particular organism to be enriched. A general discussion and recipes of non-selective media are described in the FDA Bacteriological Analytical Manual. (1998) published and distributed by the Association of Analytical Chemists, Suite 400, 2200 Wilson Blvd, Arlington, VA 22201-3301.

After selective growth, a sample of the complex mixtures is removed for further analysis. This sampling procedure may be accomplished by a variety of means well known to those skilled in the art. In a preferred embodiment, 5 µl of the enrichment culture is removed and added to 200 µl of lysis solution containing protease. The lysis solution is heated at 37°C for 20 min followed by protease inactivation at 95°C for 10 min as described in the BAX® System User's Guide, DuPont Qualicon, Inc., Wilmington, DE.

**PCR Assay Methods:**

A preferred method for detecting the presence of the present invention's target nucleic acids and subsequently *Salmonella* in a sample comprises (a) performing PCR amplification using a primer and probe, or a primer-probe complex, of the present invention, such as those described in Figs. 1A-1C, and a suitable reverse primer; and (b) detecting the amplification, whereby a positive detection of the amplification indicates the presence of *Salmonella* in the sample. In another embodiment, PCR amplification is performed using two different primer-probe complexes of the present invention that have primer binding regions that are sufficiently separated such that one primer-probe complex acts as a forward primer and the second primer-probe complex acts as a reverse primer. Looking at Figs. 1A-1C, examples of such forward-acting primer-probe complexes include S35C610-1, S35C61 0-2, S35C61 0-2a, S35C61 0-3a, S35C61 0-3a, S35C610-3a, S35C61 0-3b, S35C61 0-4b, S35C61 0-5b, S35FAM-3a, S35Q670-2a, S35TEX1, S35TEX1a, S35TEX2, S35TEX2, S35TEX2b, S35TEX2c, S35TEX3, S35TEX3a, S35TEX3a,
S35TEX-3a, and SB35C61 0, while examples of such reverse-acting primer-probe complexes include S761 aC61 0-4d, S761 bC61 0-4g, S761 bC61 0-5g, S761 C610-3, S761 C61 0-3a, S761 C61 0-3b, S761 C61 0-3c, S761 C61 0-4c, S761 C61 0-4d, S761 C61 0-4e, S761 C61 0-4f, S761 C61 0-4g, S761 C61 0-5f, S761 C61 0-5g, SB761 C61 0, and SB761 C61 0-g.

In another preferred embodiment, prior to performing PCR amplification, a step of preparing the sample may be carried out. The preparing step may comprise at least one of the following processes: (1) bacterial enrichment, (2) separation of bacterial cells from the sample, (3) cell lysis, and (4) total DNA extraction.

Amplification Conditions:

A skilled person will understand that any generally acceptable PCR conditions may be used for successfully detecting the nucleic acid targets and the target Salmonella bacteria using the oligonucleotides of the instant invention, and depending on the sample to be tested and other laboratory conditions, routine optimization for the PCR conditions may be necessary to achieve optimal sensitivity and specificity.

Detection/Examination/Analysis:

Primer-directed amplification products produced by the methods of the present invention can be analyzed using various methods. Homogenous detection refers to a preferred method for the detection of amplification products where no separation (such as by gel electrophoresis) of amplification products from template or primers is necessary. Homogeneous detection is typically accomplished by measuring the level of fluorescence of the reaction mixture during or immediately following amplification. In addition, heterogeneous detection methods, which involve separation of amplification products during or prior to detection, can be employed in the present invention.

Homogenous detection may be employed to carry out "real-time" primer-directed nucleic acid amplification and detection, using primer-probe complexes of the instant invention (e.g., "real-time" PCR and "real-time" RT-PCR). A particularly
preferred "real-time" detection method is the Scorpion probe assay as set forth in U.S. Patent No. 6,326,145, which is hereby incorporated by reference in its entirety. In the Scorpion probe assay, PCR amplification is performed using a Scorpion probe (either unimolecular or bimolecular) as a primer-probe complex, the Scorpion probe possessing an appropriate reporter-quencher pair to allow the detectable signal of the reporter to be quenched prior to elongation of the primer. Post-elongation, the quenching effect is eliminated and the amount of signal present is quantitated. As the amount of amplification product increases, an equivalent increase in detectable signal will be observed, thus allowing the amount of amplification product present to be determined as a function of the amount of detectable signal measured. When more than one Scorpion probe is employed in a Scorpion probe assay of present invention, each probe can have the same detectable label attached or a different detectable label attached, thus allowing each probe to be detected independently of the other probes.

Another preferred "real-time" detection method is the 5'-exonuclease detection method, as set forth in U.S. Patent Nos. 5,804,375, 5,538,848, 5,487,972, and 5,210,015, each of which is hereby incorporated by reference in its entirety. In the 5'-exonuclease detection assay, a modified probe is employed during PCR that binds intermediate to or between the two members of an amplification primer pair. The modified probe possesses a reporter and a quencher and is designed to generate a detectable signal to indicate that it has hybridized with the target nucleic acid sequence during PCR. As long as both the reporter and the quencher are on the probe, the quencher stops the reporter from emitting a detectable signal. However, as the polymerase extends the primer during amplification, the intrinsic 5' to 3' nuclease activity of the polymerase degrades the probe, separating the reporter from the quencher, and enabling the detectable signal to be emitted. Generally, the amount of detectable signal generated during the amplification cycle is proportional to the amount of product generated in each cycle.

It is well known that the efficiency of quenching is a strong function of the proximity of the reporter and the quencher, i.e., as the two molecules get closer, the quenching efficiency increases. As quenching is strongly dependent on the physical
proximity of the reporter and quencher, the reporter and the quencher are preferably attached to the probe within a few nucleotides of one another, usually within 30 nucleotides of one another, more preferably with a separation of from about 6 to 16 nucleotides. Typically, this separation is achieved by attaching one member of a reporter-quencher pair to the 5' end of the probe and the other member to a nucleotide about 6 to 16 nucleotides away.

Again, when more than one TaqMan® probe is employed in a 5'-exonuclease detection assay of present invention, such as one directed to two or more of SEQ ID NOs: 686-696, each probe can have a different detectable label (e.g., reporter-quencher pair) attached, thus allowing each probe to be detected independently of the other probes.

In addition to homogenous detection methods, a variety of other heterogeneous detection methods are known in the art that can be employed in the present invention, including standard non-denaturing gel electrophoresis (e.g., acrylamide or agarose), denaturing gradient gel electrophoresis, and temperature gradient gel electrophoresis. Standard non-denaturing gel electrophoresis is a simple and quick method of PCR detection, but may not be suitable for all applications.

**Instrumentation:**

When homogenous detection is employed, the level of fluorescence is preferably measured using a laser fluorometer such as, for example, an ABI Prism Model 7500 Fast Sequence Detector. However, similar detection systems for measuring the level of fluorescence in a sample are included in the invention.

**Reagents and Kits:**

Any suitable nucleic acid replication composition ("replication composition") in any format can be used. A typical replication composition for PCR amplification may comprise, for example, dATP, dCTP, dGTP, and dTTP; target specific primers, probes, or primer-probe complexes; and a suitable polymerase.

If the replication composition is in liquid form, suitable buffers known in the art may be used (Sambrook, J. et al., supra).
Alternatively, if the replication composition is contained in a tablet form, then

typical tabletization reagents may be included such as stabilizers and binding agents.

Preferred tabletization technology is set forth in U.S. Patent Nos. 4,762,857 and

4,678,812, each of which is hereby incorporated by reference in its entirety.

In certain embodiments, the replication composition of the instant invention

comprises at least one primer and probe and a thermostable DNA polymerase,

wherein the primer is at least 10 nucleotides in length and the probe is at least 10

nucleotides in length, and wherein (i) the primer comprises a nucleic acid sequence

capable of selectively hybridizing under stringent conditions to the sequence of SEQ

ID NO: 1 or a sequence complementary thereto, and the probe comprises a nucleic

acid sequence capable of selectively hybridizing under stringent conditions to the

sequence of SEQ ID NO: 2 or a sequence complementary thereto; or (ii) the primer

comprises a nucleic acid sequence capable of selectively hybridizing under stringent

conditions to the nucleic acid sequence of SEQ ID NO: 3 or a sequence

complementary thereto, and the probe comprises a nucleic acid sequence capable of

selectively hybridizing under stringent conditions to the sequence of SEQ ID NO: 4 or

a sequence complementary thereto. In certain specific embodiments, the primer is at

least 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30

nucleotides in length. In further embodiments, the probe is at least 11, 12, 13, 14,

15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length.

In some examples, the primer and probe are directly or indirectly attached,

thereby forming a primer-probe complex. In other examples, the primer-probe

complex involves direct or indirect attachment of the 3' terminus of the probe to the 5'

terminus of the primer. In further examples, the probe portion of the primer-probe

complex is flanked by a 5' Stem Sequence, such as set forth in SEQ ID NOs: 112-

119, and a 3' Stem Sequence, such as set forth in SEQ ID NOs: 120-130.

In some embodiments, the primer portion capable of selectively hybridizing

under stringent conditions to the sequence of SEQ ID NO: 1 comprises a nucleic acid

sequence selected from the group consisting of SEQ ID NOs: 40-54. In other

embodiments, the primer portion capable of selectively hybridizing under stringent

conditions to the sequence of SEQ ID NO: 2 comprises a nucleic acid sequence
selected from the group consisting of SEQ ID NOs: 5-39. In further embodiments, the primer portion capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO: 3 comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 95-111. In still further embodiments, the primer portion capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO: 4 comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 55-94.

In other specific embodiments, the replication composition of the instant invention comprises (a) at least one primer-probe complex selected from Figs. 1A-1C, and (b) thermostable DNA polymerase. Another preferred replication composition comprises (a) at least two primer-probe complexes selected from Figs. 1A-1C, each directed toward a different target DNA region such that one complex acts as a forward primer and the other acts as a reverse primer; and (b) thermostable DNA polymerase. In certain examples, the forward-acting primer-probe complex is selected from the group consisting of S35C61 0-1, S35C61 0-2, S35C61 0-2a, S35C61 0-3a, S35C610-3a, S35C61 0-3a, S35C610-4b, S35C61 0-5b, S35FAM-3a, S35Q670-2a, S35TEX1, S35TEX1a, S35TEX2, S35TEX2b, S35TEX2c, S35TEX3, S35TEX3a, S35TEX3a, S35TEX-3a, and SB35C61 0, while the reverse-acting primer-probe complex is selected from the group consisting of S761 aC61 0-4d, S761 bC61 0-4g, S761 bC61 0-5g, S761 C61 0-3, S761 C61 0-3a, S761 C61 0-3b, S761 C61 0-3c, S761 C61 0-4c, S761 C610-4d, S761 C61 0-4e, S761 C61 0-4f, S761 C61 0-4g, S761 C61 0-5f, S761 C61 0-5g, SB761 C61 0, and SB761 C61 0-g. In certain examples, the replication composition further comprises a suitable quencher oligonucleotide capable of binding to, and quenching the signal of, the probe portion of the primer-probe complex.

A preferred kit of the instant invention comprises any one of the above replication compositions. A preferred tablet of the instant invention comprises any one of the above replication compositions. More preferably, a kit of the instant invention comprises the foregoing preferred tablet.

In some instances, an internal positive control can be included in the reaction. The internal positive control can include control template nucleic acids (e.g. DNA or
RNA), control primers, and a control nucleic acid probe. The advantages of an internal positive control contained within a PCR reaction have been previously described (U.S. Patent No. 6,312,930 and PCT Application No. WO 97/11197, each of which is hereby incorporated by reference in its entirety), and include: (i) the control may be amplified using a single primer; (ii) the amount of the control amplification product is independent of any target DNA or RNA contained in the sample; (iii) the control DNA can be tableted with other amplification reagents for ease of use and high degree of reproducibility in both manual and automated test procedures; (iv) the control can be used with homogeneous detection, i.e., without separation of product DNA from reactants; and (v) the internal control has a melting profile that is distinct from other potential amplification products in the reaction and/or a detectable label on the control nucleic acid that is distinct from the detectable label on the nucleic acid probe directed to the target.

Control DNA will be of appropriate size and base composition to permit amplification in a primer-directed amplification reaction. The control template DNA sequence may be obtained from any suitable source, but must be reproducibly amplified under the same conditions that permit the amplification of the target amplification product.

Preferred control sequences include, for example, control primers and probes directed toward SV40 DNA.

The control reaction is useful to validate the amplification reaction. Amplification of the control DNA occurs within the same reaction tube as the sample that is being tested, and therefore indicates a successful amplification reaction when samples are target negative, i.e. no target amplification product is produced. In order to achieve significant validation of the amplification reaction, a suitable number of copies of the control DNA template must be included in each amplification reaction.

In some instances, it may be useful to include an additional negative control replication composition. The negative control replication composition will contain the same reagents as the replication composition but without the polymerase. The primary function of such a control is to monitor spurious background fluorescence in a homogeneous format when the method employs a fluorescent means of detection.
Replication compositions may be modified depending on whether they are designed to be used to amplify target DNA or the control DNA. Replication compositions that will amplify the target DNA (test replication compositions) may include (i) a polymerase (generally thermostable), (ii) a primer pair capable of hybridizing to the target DNA and (iii) necessary buffers for the amplification reaction to proceed. Replication compositions that will amplify the control DNA (positive control, or positive replication composition) may include (i) a polymerase (generally thermostable) (ii) the control DNA; (iii) at least one primer capable of hybridizing to the control DNA; and (iv) necessary buffers for the amplification reaction to proceed. In addition, the replication composition for either target DNA or control DNA amplification can contain a nucleic acid probe, preferably possessing a detectable label.

EXAMPLES

The present invention is further defined in the following Examples. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only.

EXAMPLE 1

Determination of inclusivity/exclusivity of the individual targets via Scorpion® assay

Samples of organisms were analyzed to establish inclusivity and exclusivity of numerous Scorpion® probes of the present invention. For inclusivity, independent, bona fide Salmonella isolates were used. For exclusivity, closely related non-target organisms were used to ensure that the assay would discriminate the target organisms from other non-target organisms.

DNA lysate preparation

Material tested was overnight growth pure cultures of the target and non-target organisms grown at 37 °C in BHI media. Pure cultures grown overnight to cell densities of approximately 1x10^9 cfu/ml. For exclusivity, 1:10 dilutions of overnight cultures were tested. For inclusivity, overnight cultures were diluted approximately 1:1,000 into TSB. 5 µl of the material to be tested was added to 200 µl of BAX®
lysis reagent (DuPont Qualicon, Wilmington, DE). The mixture was incubated at 37 °C for 20 minutes, then further incubated at 95 °C for 10 minutes, and finally cooled to 5 °C.

5 **PCR conditions**

30 µl of the DNA lysate was used to hydrate lyophilized PCR reaction components to obtain DNA lysate/PCR reaction component mixtures. The PCR reaction components were in the form of customized reagent containing GoTaq DNA Polymerase (Promega, Madison, WI), deoxynucleotides (Roche Diagnostics, Indianapolis, IN), BSA, and surfactamps (Sigma-Aldrich, St. Louis, MO). In addition, the primers and Scorpion® probes listed in Table 1 were included in the amounts provided. As this Table demonstrates, each of these Scorpion® probes was designed as a uni-molecular Scorpion®, such that its structure includes (in 5' to 3' order): a 5' fluorescent end label, 5' Stem Sequence, a probe sequence, a 3' Stem Sequence, an internal quencher, an 18-carbon non-amplifiable linker, and a primer sequence.

<table>
<thead>
<tr>
<th>Nucleotide Name</th>
<th>Target</th>
<th>Amt. Per Rxn.</th>
<th>5' End Label</th>
<th>5' Stem SEQ ID NO:</th>
<th>Probe SEQ ID NO:</th>
<th>3' Stem SEQ ID NO:</th>
<th>Internal Label / Linker</th>
<th>Primer SEQ ID NO:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward Scorpion S35C610-3a</td>
<td>Salmonella</td>
<td>50-150 nM</td>
<td>Cal Fluor Red 610</td>
<td>114</td>
<td>29</td>
<td>124</td>
<td>BHQ2 / 18-Carbon Linker</td>
<td>47</td>
</tr>
<tr>
<td>Forward Primer 35</td>
<td>Salmonella</td>
<td>250-350 nM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>47</td>
</tr>
<tr>
<td>Reverse Scorpion S761C610-4d</td>
<td>Salmonella</td>
<td>50-150 nM</td>
<td>Cal Fluor Red 610</td>
<td>116</td>
<td>90</td>
<td>127</td>
<td>BHQ2 / 18-Carbon Linker</td>
<td>111</td>
</tr>
<tr>
<td>Reverse Scorpion S761C610-5g</td>
<td>Salmonella</td>
<td>50-150 nM</td>
<td>Cal Fluor Red 610</td>
<td>117</td>
<td>93</td>
<td>128</td>
<td>BHQ2 / 18-Carbon Linker</td>
<td>102</td>
</tr>
<tr>
<td>Reverse Primer 761</td>
<td>Salmonella</td>
<td>250-350 nM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>111</td>
</tr>
<tr>
<td>Forward Scorpion S4219EC560-3b</td>
<td>Positive Control (sSV40)</td>
<td>10-50 nM</td>
<td>Cal Fluor Orange 560</td>
<td>132</td>
<td>133</td>
<td>134</td>
<td>BHQ1 / 18-Carbon Linker</td>
<td>135</td>
</tr>
</tbody>
</table>
Amplification and testing were performed on the BAX® Q7 instrument (DuPont Qualicon, Wilmington, DE). The thermal cycling conditions were as follows: 2 minutes at 95 °C, followed by 46 cycles of 95 °C for 10 seconds and 70 °C for 50 seconds, with the fluorescent signal captured during the 70 °C step at each cycle.

Results

As can be seen in Tables 2-3, using Scorpion® probes, the method of the present invention was able to detect the various targets appropriately, including distinguishing between target and non-target organisms.

### TABLE 2. Inclusivity Results

<table>
<thead>
<tr>
<th>DuPont Qualicon ID #</th>
<th>Presumptive ID</th>
<th>ID Species</th>
<th>BAX® System Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>584</td>
<td>Salmonella typhi</td>
<td>Salmonella typhi</td>
<td>Positive</td>
</tr>
<tr>
<td>585</td>
<td>Salmonella typhi</td>
<td>Salmonella typhi</td>
<td>Positive</td>
</tr>
<tr>
<td>586</td>
<td>Salmonella typhimurium</td>
<td>Salmonella typhimurium</td>
<td>Positive</td>
</tr>
<tr>
<td>706</td>
<td>Salmonella enteritidis</td>
<td>Salmonella enteritidis</td>
<td>Positive</td>
</tr>
<tr>
<td>707</td>
<td>Salmonella newport</td>
<td>Salmonella newport</td>
<td>Positive</td>
</tr>
<tr>
<td>725</td>
<td>Salmonella arizonae</td>
<td>Salmonella arizonae</td>
<td>Positive</td>
</tr>
<tr>
<td>726</td>
<td>Salmonella arizonae</td>
<td>Salmonella arizonae</td>
<td>Positive</td>
</tr>
<tr>
<td>737</td>
<td>Salmonella enteritidis</td>
<td>Salmonella enteritidis</td>
<td>Positive</td>
</tr>
<tr>
<td>738</td>
<td>Salmonella virchow</td>
<td>Salmonella virchow</td>
<td>Positive</td>
</tr>
<tr>
<td>739</td>
<td>Salmonella stanley</td>
<td>Salmonella stanley</td>
<td>Positive</td>
</tr>
<tr>
<td>741</td>
<td>Salmonella gallinarum</td>
<td>Salmonella gallinarum</td>
<td>Positive</td>
</tr>
<tr>
<td>917</td>
<td>Salmonella choleraesuis</td>
<td>Salmonella choleraesuis</td>
<td>Positive</td>
</tr>
<tr>
<td>918</td>
<td>Salmonella paratyphi A</td>
<td>Salmonella paratyphi</td>
<td>Positive</td>
</tr>
<tr>
<td>919</td>
<td>Salmonella paratyphi A</td>
<td>Salmonella paratyphi</td>
<td>Positive</td>
</tr>
<tr>
<td>964</td>
<td>Salmonella bredeney</td>
<td>Salmonella bredeney</td>
<td>Positive</td>
</tr>
<tr>
<td>966</td>
<td>Salmonella napolii</td>
<td>Salmonella napolii</td>
<td>Positive</td>
</tr>
<tr>
<td>1084</td>
<td>Salmonella typhimurium</td>
<td>Salmonella typhimurium</td>
<td>Positive</td>
</tr>
<tr>
<td>1084</td>
<td>Salmonella typhimurium</td>
<td>Salmonella typhimurium</td>
<td>Positive</td>
</tr>
<tr>
<td>1085</td>
<td>Salmonella binza</td>
<td>Salmonella binza</td>
<td>Positive</td>
</tr>
<tr>
<td>1248</td>
<td>Salmonella panama</td>
<td>Salmonella panama</td>
<td>Positive</td>
</tr>
<tr>
<td>1251</td>
<td>Salmonella kedougou</td>
<td>Salmonella kedougou</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Salmonella montevideo</td>
<td>Salmonella montevideo</td>
<td>Positive</td>
</tr>
<tr>
<td>---</td>
<td>------------------------</td>
<td>------------------------</td>
<td>----------</td>
</tr>
<tr>
<td>1255</td>
<td>Salmonella newport</td>
<td>Salmonella newport</td>
<td>Positive</td>
</tr>
<tr>
<td>1261</td>
<td>Salmonella braenderup</td>
<td>Salmonella braenderup</td>
<td>Positive</td>
</tr>
<tr>
<td>1329</td>
<td>Salmonella montevideo</td>
<td>Salmonella montevideo</td>
<td>Positive</td>
</tr>
<tr>
<td>1331</td>
<td>Salmonella anatum</td>
<td>Salmonella anatum</td>
<td>Positive</td>
</tr>
<tr>
<td>1332</td>
<td>Salmonella Stanley</td>
<td>Salmonella Stanley</td>
<td>Positive</td>
</tr>
<tr>
<td>1333</td>
<td>Salmonella anatum</td>
<td>Salmonella anatum</td>
<td>Positive</td>
</tr>
<tr>
<td>1334</td>
<td>Salmonella agona</td>
<td>Salmonella agona</td>
<td>Positive</td>
</tr>
<tr>
<td>1335</td>
<td>Salmonella thompson</td>
<td>Salmonella thompson</td>
<td>Positive</td>
</tr>
<tr>
<td>1336</td>
<td>Salmonella braenderup</td>
<td>Salmonella braenderup</td>
<td>Positive</td>
</tr>
<tr>
<td>1337</td>
<td>Salmonella braenderup</td>
<td>Salmonella braenderup</td>
<td>Positive</td>
</tr>
<tr>
<td>1338</td>
<td>Salmonella brandenburg</td>
<td>Salmonella brandenburg</td>
<td>Positive</td>
</tr>
<tr>
<td>1339</td>
<td>Salmonella thompson</td>
<td>Salmonella thompson</td>
<td>Positive</td>
</tr>
<tr>
<td>1343</td>
<td>Salmonella blockley</td>
<td>Salmonella haardt</td>
<td>Positive</td>
</tr>
<tr>
<td>1352</td>
<td>Salmonella agona</td>
<td>Salmonella agona</td>
<td>Positive</td>
</tr>
<tr>
<td>1356</td>
<td>Salmonella breedeney</td>
<td>Salmonella breedeney</td>
<td>Positive</td>
</tr>
<tr>
<td>1372</td>
<td>Salmonella saintpaul</td>
<td>Salmonella saintpaul</td>
<td>Positive</td>
</tr>
<tr>
<td>1424</td>
<td>Salmonella manchester</td>
<td>Salmonella manchester</td>
<td>Positive</td>
</tr>
<tr>
<td>1429</td>
<td>unknown</td>
<td>Salmonella anfo</td>
<td>Positive</td>
</tr>
<tr>
<td>1467</td>
<td>Salmonella typhimurium</td>
<td>Salmonella typhimurium</td>
<td>Positive</td>
</tr>
<tr>
<td>1469</td>
<td>Salmonella ealing</td>
<td>Salmonella ealing</td>
<td>Positive</td>
</tr>
<tr>
<td>1476</td>
<td>Salmonella napoli</td>
<td>Salmonella napoli</td>
<td>Positive</td>
</tr>
<tr>
<td>1480</td>
<td>Salmonella indiana</td>
<td>Salmonella indiana</td>
<td>Positive</td>
</tr>
<tr>
<td>1482</td>
<td>Salmonella pullorum</td>
<td>Salmonella pullorum</td>
<td>Positive</td>
</tr>
<tr>
<td>1491</td>
<td>Salmonella weltevreden</td>
<td>Salmonella weltevreden</td>
<td>Positive</td>
</tr>
<tr>
<td>1492</td>
<td>Salmonella montevideo</td>
<td>Salmonella montevideo</td>
<td>Positive</td>
</tr>
<tr>
<td>1507</td>
<td>Salmonella pullorum</td>
<td>Salmonella pullorum</td>
<td>Positive</td>
</tr>
<tr>
<td>1509</td>
<td>Salmonella bovismorbificans</td>
<td>Salmonella bovismorbificans</td>
<td>Positive</td>
</tr>
<tr>
<td>1510</td>
<td>Salmonella bareilly</td>
<td>Salmonella bareilly</td>
<td>Positive</td>
</tr>
<tr>
<td>1521</td>
<td>Salmonella amersfoort</td>
<td>Salmonella abaeetuba</td>
<td>Positive</td>
</tr>
<tr>
<td>1523</td>
<td>Salmonella berkeley</td>
<td>Salmonella berkeley</td>
<td>Positive</td>
</tr>
<tr>
<td>1525</td>
<td>Salmonella betioky</td>
<td>Salmonella betioky</td>
<td>Positive</td>
</tr>
<tr>
<td>1526</td>
<td>Salmonella austin</td>
<td>Salmonella austin</td>
<td>Positive</td>
</tr>
<tr>
<td>1527</td>
<td>Salmonella atlanta</td>
<td>Salmonella atlanta</td>
<td>Positive</td>
</tr>
<tr>
<td>1530</td>
<td>Salmonella amager</td>
<td>Salmonella altendorf</td>
<td>Positive</td>
</tr>
<tr>
<td>1531</td>
<td>Salmonella altendorf</td>
<td>Salmonella altendorf</td>
<td>Positive</td>
</tr>
<tr>
<td>1535</td>
<td>Salmonella brookfield</td>
<td>Salmonella brookfield</td>
<td>Positive</td>
</tr>
<tr>
<td>1543</td>
<td>Salmonella adeela</td>
<td>Salmonella adeela</td>
<td>Positive</td>
</tr>
<tr>
<td>1547</td>
<td>Salmonella aberdeen</td>
<td>Salmonella species</td>
<td>Positive</td>
</tr>
<tr>
<td>1548</td>
<td>Salmonella abony</td>
<td>Salmonella abony</td>
<td>Positive</td>
</tr>
<tr>
<td>1551</td>
<td>Salmonella aequatorium</td>
<td>Salmonella species</td>
<td>Positive</td>
</tr>
<tr>
<td>1552</td>
<td>Salmonella alabama</td>
<td>Salmonella alabama</td>
<td>Positive</td>
</tr>
<tr>
<td>1553</td>
<td>Salmonella ball</td>
<td>Salmonella ball</td>
<td>Positive</td>
</tr>
<tr>
<td>1554</td>
<td>Salmonella banalia</td>
<td>Salmonella species</td>
<td>Positive</td>
</tr>
<tr>
<td>1555</td>
<td>Salmonella branacter</td>
<td>Salmonella branacter</td>
<td>Positive</td>
</tr>
<tr>
<td>1556</td>
<td>Salmonella alachua</td>
<td>Salmonella alachua</td>
<td>Positive</td>
</tr>
<tr>
<td>1557</td>
<td>Salmonella Chicago</td>
<td>Salmonella Chicago</td>
<td>Positive</td>
</tr>
<tr>
<td>1558</td>
<td>Salmonella canastel</td>
<td>Salmonella species</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Salmonella westpark</td>
<td>Salmonella species</td>
<td>Positive</td>
</tr>
<tr>
<td>---</td>
<td>---------------------</td>
<td>--------------------</td>
<td>----------</td>
</tr>
<tr>
<td>1560</td>
<td>Salmonella</td>
<td>Salmonella</td>
<td>Positive</td>
</tr>
<tr>
<td>1566</td>
<td>Salmonella arizonae</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>1568</td>
<td>Salmonella arizonae</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>1573</td>
<td>Salmonella arizonae</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>1576</td>
<td>Salmonella arizonae</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>1585</td>
<td>Salmonella arizonae</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>1590</td>
<td>Salmonella 3b</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>1592</td>
<td>Salmonella 3b</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>1597</td>
<td>Salmonella 3b</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>1598</td>
<td>Salmonella 3b</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>1603</td>
<td>Salmonella 3b</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>1608</td>
<td>Salmonella seminole</td>
<td>Salmonella seminole</td>
<td>Positive</td>
</tr>
<tr>
<td>1609</td>
<td>Salmonella wassenaar</td>
<td>Salmonella wassenaar</td>
<td>Positive</td>
</tr>
<tr>
<td>1610</td>
<td>Salmonella seminole</td>
<td>Salmonella seminole</td>
<td>Positive</td>
</tr>
<tr>
<td>1611</td>
<td>Salmonella</td>
<td>Salmonella kralendyk</td>
<td>Positive</td>
</tr>
<tr>
<td>1613</td>
<td>Salmonella tuindorp</td>
<td>Salmonella kralendyk</td>
<td>Positive</td>
</tr>
<tr>
<td>1615</td>
<td>Salmonella chameleon</td>
<td>Salmonella kralendyk</td>
<td>Positive</td>
</tr>
<tr>
<td>1616</td>
<td>Salmonella houten</td>
<td>Salmonella houten</td>
<td>Positive</td>
</tr>
<tr>
<td>1620</td>
<td>Salmonella carmelo</td>
<td>Salmonella carmelo</td>
<td>Positive</td>
</tr>
<tr>
<td>1621</td>
<td>Salmonella carrau</td>
<td>Salmonella carrau</td>
<td>Positive</td>
</tr>
<tr>
<td>1623</td>
<td>Salmonella champaign</td>
<td>Salmonella champaign</td>
<td>Positive</td>
</tr>
<tr>
<td>1624</td>
<td>Salmonella champaign</td>
<td>Salmonella champaign</td>
<td>Positive</td>
</tr>
<tr>
<td>1625</td>
<td>Salmonella Chester</td>
<td>Salmonella species</td>
<td>Positive</td>
</tr>
<tr>
<td>1628</td>
<td>Salmonella Colorado</td>
<td>Salmonella species</td>
<td>Positive</td>
</tr>
<tr>
<td>1632</td>
<td>Salmonella cubana</td>
<td>Salmonella cubana</td>
<td>Positive</td>
</tr>
<tr>
<td>1635</td>
<td>Salmonella daytona</td>
<td>Salmonella daytona</td>
<td>Positive</td>
</tr>
<tr>
<td>1638</td>
<td>Salmonella derby</td>
<td>Salmonella derby</td>
<td>Positive</td>
</tr>
<tr>
<td>1641</td>
<td>Salmonella durban</td>
<td>Salmonella species</td>
<td>Positive</td>
</tr>
<tr>
<td>1644</td>
<td>Salmonella ealing</td>
<td>Salmonella ealing</td>
<td>Positive</td>
</tr>
<tr>
<td>1650</td>
<td>Salmonella livingstone</td>
<td>Salmonella livingstone</td>
<td>Positive</td>
</tr>
<tr>
<td>1652</td>
<td>Salmonella livingstone</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>1653</td>
<td>Salmonella manhattan</td>
<td>Salmonella yovokome</td>
<td>Positive</td>
</tr>
<tr>
<td>1655</td>
<td>Salmonella reading</td>
<td>Salmonella reading</td>
<td>Positive</td>
</tr>
<tr>
<td>1657</td>
<td>Salmonella reading</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>1658</td>
<td>Salmonella Schwarzengrund</td>
<td>Salmonella Schwarzengrund</td>
<td>Positive</td>
</tr>
<tr>
<td>1659</td>
<td>Salmonella shangani</td>
<td>Salmonella shangani</td>
<td>Positive</td>
</tr>
<tr>
<td>1660</td>
<td>Salmonella sundsvall</td>
<td>Salmonella sundsvall</td>
<td>Positive</td>
</tr>
<tr>
<td>1661</td>
<td>Salmonella tennesseee</td>
<td>Salmonella tennesseee</td>
<td>Positive</td>
</tr>
<tr>
<td>1665</td>
<td>Salmonella Colombo</td>
<td>Salmonella Colombo</td>
<td>Positive</td>
</tr>
<tr>
<td>1668</td>
<td>Salmonella California</td>
<td>Salmonella California</td>
<td>Positive</td>
</tr>
<tr>
<td>1675</td>
<td>Salmonella daressalaam</td>
<td>Salmonella enterica</td>
<td>Positive</td>
</tr>
<tr>
<td>1680</td>
<td>Salmonella dugbe</td>
<td>Salmonella dugbe</td>
<td>Positive</td>
</tr>
<tr>
<td>1684</td>
<td>Salmonella emmastad</td>
<td>Salmonella emmastad</td>
<td>Positive</td>
</tr>
<tr>
<td>1686</td>
<td>Salmonella fayed</td>
<td>Salmonella fayed</td>
<td>Positive</td>
</tr>
<tr>
<td>1687</td>
<td>Salmonella ferylac</td>
<td>Salmonella ferylac</td>
<td>Positive</td>
</tr>
<tr>
<td>1689</td>
<td>Salmonella hartford</td>
<td>Salmonella species</td>
<td>Positive</td>
</tr>
<tr>
<td>1693</td>
<td>Salmonella javiana</td>
<td>Salmonella species</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Salmonella Johannesburg</td>
<td>Salmonella Johannesburg</td>
<td>Positive</td>
</tr>
<tr>
<td>---</td>
<td>-------------------------</td>
<td>-------------------------</td>
<td>----------</td>
</tr>
<tr>
<td>1695</td>
<td>Salmonella madelia</td>
<td>Salmonella madelia</td>
<td>Positive</td>
</tr>
<tr>
<td>1698</td>
<td>Salmonella meleagris</td>
<td>Salmonella meleagris</td>
<td>Positive</td>
</tr>
<tr>
<td>1700</td>
<td>Salmonella miami</td>
<td>Salmonella miami</td>
<td>Positive</td>
</tr>
<tr>
<td>1701</td>
<td>Salmonella mississippi</td>
<td>Salmonella mississippi</td>
<td>Positive</td>
</tr>
<tr>
<td>1703</td>
<td>Salmonella muenchen</td>
<td>Salmonella muenchen</td>
<td>Positive</td>
</tr>
<tr>
<td>1704</td>
<td>Salmonella newbrunswick</td>
<td>Salmonella newbrunswick</td>
<td>Positive</td>
</tr>
<tr>
<td>1707</td>
<td>Salmonella oranienburg</td>
<td>Salmonella oranienburg</td>
<td>Positive</td>
</tr>
<tr>
<td>1710</td>
<td>Salmonella pomona</td>
<td>Salmonella species</td>
<td>Positive</td>
</tr>
<tr>
<td>1711</td>
<td>Salmonella pretoria</td>
<td>Salmonella pretoria</td>
<td>Positive</td>
</tr>
<tr>
<td>1712</td>
<td>Salmonella wassennaar</td>
<td>Salmonella wassennaar</td>
<td>Positive</td>
</tr>
<tr>
<td>1714</td>
<td>Salmonella enterica</td>
<td>Salmonella brookfield</td>
<td>Positive</td>
</tr>
<tr>
<td>1717</td>
<td>Salmonella enterica</td>
<td>Salmonella typhimurium</td>
<td>Positive</td>
</tr>
<tr>
<td>1717</td>
<td>Salmonella enterica</td>
<td>Salmonella kralendyk</td>
<td>Positive</td>
</tr>
<tr>
<td>1717</td>
<td>Salmonella enterica</td>
<td>Salmonella enterica</td>
<td>Positive</td>
</tr>
<tr>
<td>2166</td>
<td>Salmonella abaatetuba</td>
<td>Salmonella abaatetuba</td>
<td>Positive</td>
</tr>
<tr>
<td>2172</td>
<td>Salmonella bareilly</td>
<td>Salmonella bareilly</td>
<td>Positive</td>
</tr>
<tr>
<td>2178</td>
<td>Salmonella California</td>
<td>Salmonella California</td>
<td>Positive</td>
</tr>
<tr>
<td>2180</td>
<td>Salmonella champaign</td>
<td>Salmonella champaign</td>
<td>Positive</td>
</tr>
<tr>
<td>2186</td>
<td>Salmonella drypool</td>
<td>Salmonella drypool</td>
<td>Positive</td>
</tr>
<tr>
<td>2189</td>
<td>Salmonella give</td>
<td>Salmonella give</td>
<td>Positive</td>
</tr>
<tr>
<td>2196</td>
<td>Salmonella kiambu</td>
<td>Salmonella kiambu</td>
<td>Positive</td>
</tr>
<tr>
<td>2199</td>
<td>Salmonella lexington</td>
<td>Salmonella lexington</td>
<td>Positive</td>
</tr>
<tr>
<td>2201</td>
<td>Salmonella madelia</td>
<td>Salmonella madelia</td>
<td>Positive</td>
</tr>
<tr>
<td>2204</td>
<td>Salmonella minnesota</td>
<td>Salmonella minnesota</td>
<td>Positive</td>
</tr>
<tr>
<td>2205</td>
<td>Salmonella mississippi</td>
<td>Salmonella mississippi</td>
<td>Positive</td>
</tr>
<tr>
<td>2215</td>
<td>Salmonella poona</td>
<td>Salmonella poona</td>
<td>Positive</td>
</tr>
<tr>
<td>2218</td>
<td>Salmonella sandiego</td>
<td>Salmonella sandiego</td>
<td>Positive</td>
</tr>
<tr>
<td>2229</td>
<td>Salmonella theilalle</td>
<td>Salmonella oranienburg</td>
<td>Positive</td>
</tr>
<tr>
<td>2238</td>
<td>Salmonella urbana</td>
<td>Salmonella urbana</td>
<td>Positive</td>
</tr>
<tr>
<td>2245</td>
<td>Salmonella havana</td>
<td>Salmonella havana</td>
<td>Positive</td>
</tr>
<tr>
<td>2263</td>
<td>Salmonella liiie</td>
<td>Salmonella liiie</td>
<td>Positive</td>
</tr>
<tr>
<td>2274</td>
<td>Salmonella anatum</td>
<td>Salmonella anatum</td>
<td>Positive</td>
</tr>
<tr>
<td>2283</td>
<td>Salmonella newbrunswick</td>
<td>Salmonella newbrunswick</td>
<td>Positive</td>
</tr>
<tr>
<td>2289</td>
<td>Salmonella rubislaw</td>
<td>Salmonella rubislaw</td>
<td>Positive</td>
</tr>
<tr>
<td>2290</td>
<td>Salmonella hartford</td>
<td>Salmonella hartford</td>
<td>Positive</td>
</tr>
<tr>
<td>2309</td>
<td>Salmonella maregrosso</td>
<td>Salmonella species</td>
<td>Positive</td>
</tr>
<tr>
<td>2312</td>
<td>Salmonella kottbus</td>
<td>Salmonella kottbus</td>
<td>Positive</td>
</tr>
<tr>
<td>2313</td>
<td>Salmonella wandsworth</td>
<td>Salmonella wandsworth</td>
<td>Positive</td>
</tr>
<tr>
<td>2341</td>
<td>Salmonella barry</td>
<td>Salmonella mbandaka</td>
<td>Positive</td>
</tr>
<tr>
<td>2343</td>
<td>Salmonella bockenhein</td>
<td>Salmonella kralendyk</td>
<td>Positive</td>
</tr>
<tr>
<td>2346</td>
<td>Salmonella Vietnam</td>
<td>Salmonella Vietnam</td>
<td>Positive</td>
</tr>
<tr>
<td>2349</td>
<td>Salmonella drypool</td>
<td>Salmonella drypool</td>
<td>Positive</td>
</tr>
<tr>
<td>2350</td>
<td>Salmonella gallinarum</td>
<td>Salmonella gallinarum</td>
<td>Positive</td>
</tr>
<tr>
<td>2352</td>
<td>Salmonella saphra</td>
<td>Salmonella saphra</td>
<td>Positive</td>
</tr>
<tr>
<td>2353</td>
<td>Salmonella kristianstad</td>
<td>Salmonella kristianstad</td>
<td>Positive</td>
</tr>
<tr>
<td>2373</td>
<td>Salmonella species</td>
<td>Salmonella species</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Salmonella species</td>
<td>Salmonella species</td>
<td>Positive</td>
</tr>
<tr>
<td>---</td>
<td>-------------------</td>
<td>-------------------</td>
<td>---------</td>
</tr>
<tr>
<td>2376</td>
<td>Salmonella species</td>
<td>Salmonella sculcoates</td>
<td>Positive</td>
</tr>
<tr>
<td>2380</td>
<td>Salmonella species</td>
<td>Salmonella sya</td>
<td>Positive</td>
</tr>
<tr>
<td>2628</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>2629</td>
<td>Salmonella cerro</td>
<td>Salmonella cerro</td>
<td>Positive</td>
</tr>
<tr>
<td>2637</td>
<td>Salmonella schwarzengrund</td>
<td>Salmonella schwarzengrund</td>
<td>Positive</td>
</tr>
<tr>
<td>2639</td>
<td>Salmonella thomassville</td>
<td>Salmonella thomassville</td>
<td>Positive</td>
</tr>
<tr>
<td>2641</td>
<td>Salmonella schwarzengrund</td>
<td>Salmonella schwarzengrund</td>
<td>Positive</td>
</tr>
<tr>
<td>2673</td>
<td>Salmonella manhattan</td>
<td>Salmonella manhattan</td>
<td>Positive</td>
</tr>
<tr>
<td>2736</td>
<td>Salmonella oranienburg</td>
<td>Salmonella oranienburg</td>
<td>Positive</td>
</tr>
<tr>
<td>2748</td>
<td>Salmonella muenster</td>
<td>Salmonella muenster</td>
<td>Positive</td>
</tr>
<tr>
<td>2755</td>
<td>Salmonella mbandaka</td>
<td>Salmonella mbandaka</td>
<td>Positive</td>
</tr>
<tr>
<td>2757</td>
<td>Salmonella mbandaka</td>
<td>Salmonella mbandaka</td>
<td>Positive</td>
</tr>
<tr>
<td>2761</td>
<td>Salmonella mbandaka</td>
<td>Salmonella mbandaka</td>
<td>Positive</td>
</tr>
<tr>
<td>2766</td>
<td>Salmonella mbandaka</td>
<td>Salmonella mbandaka</td>
<td>Positive</td>
</tr>
<tr>
<td>2770</td>
<td>Salmonella mbandaka</td>
<td>Salmonella mbandaka</td>
<td>Positive</td>
</tr>
<tr>
<td>2774</td>
<td>Salmonella mbandaka</td>
<td>Salmonella mbandaka</td>
<td>Positive</td>
</tr>
<tr>
<td>2779</td>
<td>Salmonella mbandaka</td>
<td>Salmonella mbandaka</td>
<td>Positive</td>
</tr>
<tr>
<td>2786</td>
<td>Salmonella binza</td>
<td>Salmonella binza</td>
<td>Positive</td>
</tr>
<tr>
<td>2795</td>
<td>Salmonella berta</td>
<td>Salmonella berta</td>
<td>Positive</td>
</tr>
<tr>
<td>2813</td>
<td>Salmonella cerro</td>
<td>Salmonella cerro</td>
<td>Positive</td>
</tr>
<tr>
<td>2820</td>
<td>Salmonella braenderup</td>
<td>Salmonella braenderup</td>
<td>Positive</td>
</tr>
<tr>
<td>2867</td>
<td>Salmonella sya</td>
<td>Salmonella sya</td>
<td>Positive</td>
</tr>
<tr>
<td>2868</td>
<td>Salmonella liii</td>
<td>Salmonella Nile</td>
<td>Positive</td>
</tr>
<tr>
<td>2869</td>
<td>Salmonella durham</td>
<td>Salmonella durham</td>
<td>Positive</td>
</tr>
<tr>
<td>2870</td>
<td>Salmonella corvallis</td>
<td>Salmonella bellevue</td>
<td>Positive</td>
</tr>
<tr>
<td>2935</td>
<td>Salmonella sandiego</td>
<td>Salmonella sandiego</td>
<td>Positive</td>
</tr>
<tr>
<td>2966</td>
<td>Salmonella albany</td>
<td>Salmonella albany</td>
<td>Positive</td>
</tr>
<tr>
<td>2980</td>
<td>Salmonella arkansas</td>
<td>Salmonella arkansas</td>
<td>Positive</td>
</tr>
<tr>
<td>2981</td>
<td>Salmonella arkansas</td>
<td>Salmonella arkansas</td>
<td>Positive</td>
</tr>
<tr>
<td>2992</td>
<td>Salmonella liii</td>
<td>Salmonella Nile</td>
<td>Positive</td>
</tr>
<tr>
<td>3015</td>
<td>Salmonella dublin</td>
<td>Salmonella dublin</td>
<td>Positive</td>
</tr>
<tr>
<td>3017</td>
<td>Salmonella dublin</td>
<td>Salmonella dublin</td>
<td>Positive</td>
</tr>
<tr>
<td>3019</td>
<td>Salmonella dublin</td>
<td>Salmonella dublin</td>
<td>Positive</td>
</tr>
<tr>
<td>3038</td>
<td>Salmonella krefeld</td>
<td>Salmonella krefeld</td>
<td>Positive</td>
</tr>
<tr>
<td>3043</td>
<td>Salmonella Johannesburg</td>
<td>Salmonella Johannesburg</td>
<td>Positive</td>
</tr>
<tr>
<td>3153</td>
<td>Salmonella chandans</td>
<td>Salmonella chandans</td>
<td>Positive</td>
</tr>
<tr>
<td>3156</td>
<td>Salmonella muenchen</td>
<td>Salmonella muenchen</td>
<td>Positive</td>
</tr>
<tr>
<td>3157</td>
<td>Salmonella corvallis</td>
<td>Salmonella bellevue</td>
<td>Positive</td>
</tr>
<tr>
<td>3184</td>
<td>Salmonella sculcoates</td>
<td>Salmonella sculcoates</td>
<td>Positive</td>
</tr>
<tr>
<td>3185</td>
<td>Salmonella bellevue</td>
<td>Salmonella bellevue</td>
<td>Positive</td>
</tr>
<tr>
<td>3186</td>
<td>Salmonella sya</td>
<td>Salmonella sya</td>
<td>Positive</td>
</tr>
<tr>
<td>3187</td>
<td>Salmonella durham</td>
<td>Salmonella durham</td>
<td>Positive</td>
</tr>
<tr>
<td>3194</td>
<td>Salmonella Stanleyville</td>
<td>Salmonella Stanleyville</td>
<td>Positive</td>
</tr>
<tr>
<td>3217</td>
<td>Salmonella cotham</td>
<td>Salmonella cotham</td>
<td>Positive</td>
</tr>
<tr>
<td>3218</td>
<td>Salmonella agama</td>
<td>Salmonella agama</td>
<td>Positive</td>
</tr>
<tr>
<td>3432</td>
<td>Salmonella amager</td>
<td>Salmonella amager</td>
<td>Positive</td>
</tr>
<tr>
<td>ID</td>
<td>Strain Name</td>
<td>Species</td>
<td>Result</td>
</tr>
<tr>
<td>-----</td>
<td>----------------------</td>
<td>------------------</td>
<td>---------</td>
</tr>
<tr>
<td>351</td>
<td>Salmonella oslo</td>
<td>unknown</td>
<td>Positive</td>
</tr>
<tr>
<td>3536</td>
<td>Salmonella tennessee</td>
<td>Salmonella tennessee</td>
<td>Positive</td>
</tr>
<tr>
<td>3536</td>
<td>Salmonella hvittingfoss</td>
<td>Salmonella species</td>
<td>Positive</td>
</tr>
<tr>
<td>3699</td>
<td>Salmonella indiana</td>
<td>Salmonella indiana</td>
<td>Positive</td>
</tr>
<tr>
<td>3699</td>
<td>Salmonella othmarschen</td>
<td>Salmonella oranienburg</td>
<td>Positive</td>
</tr>
<tr>
<td>3852</td>
<td>Salmonella mbandaka</td>
<td>Salmonella mbandaka</td>
<td>Positive</td>
</tr>
<tr>
<td>3863</td>
<td>Salmonella neumuenster</td>
<td>Salmonella minnesota</td>
<td>Positive</td>
</tr>
<tr>
<td>3898</td>
<td>Salmonella hongkong</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>3917</td>
<td>Salmonella thompson</td>
<td>Salmonella thompson</td>
<td>Positive</td>
</tr>
<tr>
<td>3918</td>
<td>Salmonella harford</td>
<td>Salmonella harford</td>
<td>Positive</td>
</tr>
<tr>
<td>3924</td>
<td>Salmonella thompson</td>
<td>Salmonella thompson</td>
<td>Positive</td>
</tr>
<tr>
<td>3988</td>
<td>Salmonella enteritidis</td>
<td>Salmonella enteritidis</td>
<td>Positive</td>
</tr>
<tr>
<td>3988</td>
<td>Salmonella waycross</td>
<td>Salmonella waycross</td>
<td>Positive</td>
</tr>
<tr>
<td>4006</td>
<td>Salmonella livingstone</td>
<td>Salmonella livingstone</td>
<td>Positive</td>
</tr>
<tr>
<td>4043</td>
<td>Salmonella morton</td>
<td>Salmonella morton</td>
<td>Positive</td>
</tr>
<tr>
<td>4084</td>
<td>Salmonella kubacha</td>
<td>Salmonella kubacha</td>
<td>Positive</td>
</tr>
<tr>
<td>4102</td>
<td>Salmonella saintpaul</td>
<td>Salmonella saintpaul</td>
<td>Positive</td>
</tr>
<tr>
<td>4558</td>
<td>Salmonella redlands</td>
<td>Salmonella redlands</td>
<td>Positive</td>
</tr>
<tr>
<td>5533</td>
<td>Salmonella infantis</td>
<td>Salmonella infantis</td>
<td>Positive</td>
</tr>
<tr>
<td>5908</td>
<td>Salmonella ferlac</td>
<td>Salmonella ferlac</td>
<td>Positive</td>
</tr>
<tr>
<td>6177</td>
<td>Salmonella species</td>
<td>Salmonella species</td>
<td>Positive</td>
</tr>
<tr>
<td>6250</td>
<td>Salmonella Santiago</td>
<td>Salmonella Santiago</td>
<td>Positive</td>
</tr>
<tr>
<td>6586</td>
<td>Salmonella Santiago</td>
<td>Salmonella Santiago</td>
<td>Positive</td>
</tr>
<tr>
<td>6667</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>6696</td>
<td>Salmonella species</td>
<td>Salmonella species</td>
<td>Positive</td>
</tr>
<tr>
<td>6729</td>
<td>Salmonella manila</td>
<td>Salmonella manila</td>
<td>Positive</td>
</tr>
<tr>
<td>6735</td>
<td>Salmonella species</td>
<td>Salmonella species</td>
<td>Positive</td>
</tr>
<tr>
<td>6966</td>
<td>Salmonella cotham</td>
<td>Salmonella cotham</td>
<td>Positive</td>
</tr>
<tr>
<td>7005</td>
<td>Salmonella dublin</td>
<td>Salmonella dublin</td>
<td>Positive</td>
</tr>
<tr>
<td>7061</td>
<td>Salmonella kubacha</td>
<td>Salmonella kubacha</td>
<td>Positive</td>
</tr>
<tr>
<td>7062</td>
<td>Salmonella kubacha</td>
<td>Salmonella kubacha</td>
<td>Positive</td>
</tr>
<tr>
<td>7072</td>
<td>Salmonella amsteram</td>
<td>Salmonella amsteram</td>
<td>Positive</td>
</tr>
<tr>
<td>711</td>
<td>Salmonella infantis</td>
<td>Salmonella infantis</td>
<td>Positive</td>
</tr>
<tr>
<td>8034</td>
<td>Salmonella species</td>
<td>Salmonella species</td>
<td>Positive</td>
</tr>
<tr>
<td>1224</td>
<td>Salmonella species</td>
<td>Salmonella species</td>
<td>Positive</td>
</tr>
<tr>
<td>12904</td>
<td>Salmonella tranorora</td>
<td>Salmonella tranorora</td>
<td>Positive</td>
</tr>
<tr>
<td>12907</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>12908</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>12909</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>12910</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>12911</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>12912</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>12913</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>12914</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>1291 5</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>-------</td>
<td>---------------------</td>
<td>---------------------</td>
<td>----------</td>
</tr>
<tr>
<td>1291 6</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>1291 7</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>1291 8</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>1291 9</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>1292 0</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>1292 1</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>1292 2</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>1292 5</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>1292 6</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>1292 7</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>1292 8</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>1292 9</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>1293 1</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>1293 2</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>1293 3</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>1293 6</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>1293 7</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>1294 1</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>1294 3</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>1294 5</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>1294 6</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>1294 7</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>1294 8</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>1294 9</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>1295 0</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>1295 1</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>1295 2</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>1295 3</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>1295 4</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>1295 5</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>1295 6</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>1295 7</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>1295 9</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>1296 0</td>
<td>Salmonella senftenberg</td>
<td>Salmonella senftenberg</td>
<td>Positive</td>
</tr>
<tr>
<td>1296 1</td>
<td>Salmonella senftenberg</td>
<td>Salmonella senftenberg</td>
<td>Positive</td>
</tr>
<tr>
<td>1296 2</td>
<td>Salmonella senftenberg</td>
<td>Salmonella senftenberg</td>
<td>Positive</td>
</tr>
<tr>
<td>1296 3</td>
<td>Salmonella senftenberg</td>
<td>Salmonella senftenberg</td>
<td>Positive</td>
</tr>
<tr>
<td>1296 4</td>
<td>Salmonella senftenberg</td>
<td>Salmonella senftenberg</td>
<td>Positive</td>
</tr>
<tr>
<td>1296 5</td>
<td>Salmonella senftenberg</td>
<td>Salmonella senftenberg</td>
<td>Positive</td>
</tr>
<tr>
<td>1296 6</td>
<td>Salmonella senftenberg</td>
<td>Salmonella senftenberg</td>
<td>Positive</td>
</tr>
<tr>
<td>1296 7</td>
<td>Salmonella haardt</td>
<td>Salmonella haardt</td>
<td>Positive</td>
</tr>
<tr>
<td>1296 8</td>
<td>Salmonella haardt</td>
<td>Salmonella haardt</td>
<td>Positive</td>
</tr>
<tr>
<td>1296 9</td>
<td>Salmonella haardt</td>
<td>Salmonella haardt</td>
<td>Positive</td>
</tr>
<tr>
<td>1297 0</td>
<td>Salmonella senftenberg</td>
<td>Salmonella senftenberg</td>
<td>Positive</td>
</tr>
<tr>
<td>1297 1</td>
<td>Salmonella senftenberg</td>
<td>Salmonella senftenberg</td>
<td>Positive</td>
</tr>
<tr>
<td>1297 2</td>
<td>Salmonella senftenberg</td>
<td>Salmonella senftenberg</td>
<td>Positive</td>
</tr>
<tr>
<td>1297 5</td>
<td>Salmonella senftenberg</td>
<td>Salmonella senftenberg</td>
<td>Positive</td>
</tr>
<tr>
<td>ID</td>
<td>Species</td>
<td>Species</td>
<td>Result</td>
</tr>
<tr>
<td>-------</td>
<td>--------------------------</td>
<td>--------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>12978</td>
<td>Salmonella senftenberg</td>
<td>Salmonella senftenberg</td>
<td>Positive</td>
</tr>
<tr>
<td>12980</td>
<td>Salmonella senftenberg</td>
<td>Salmonella senftenberg</td>
<td>Positive</td>
</tr>
<tr>
<td>12981</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>12982</td>
<td>Salmonella senftenberg</td>
<td>Salmonella senftenberg</td>
<td>Positive</td>
</tr>
<tr>
<td>12983</td>
<td>Salmonella senftenberg</td>
<td>Salmonella senftenberg</td>
<td>Positive</td>
</tr>
<tr>
<td>12984</td>
<td>Salmonella senftenberg</td>
<td>Salmonella senftenberg</td>
<td>Positive</td>
</tr>
<tr>
<td>12985</td>
<td>Salmonella haardt</td>
<td>Salmonella haardt</td>
<td>Positive</td>
</tr>
<tr>
<td>12986</td>
<td>Salmonella senftenberg</td>
<td>Salmonella senftenberg</td>
<td>Positive</td>
</tr>
<tr>
<td>12987</td>
<td>Salmonella senftenberg</td>
<td>Salmonella senftenberg</td>
<td>Positive</td>
</tr>
<tr>
<td>12988</td>
<td>Salmonella senftenberg</td>
<td>Salmonella senftenberg</td>
<td>Positive</td>
</tr>
<tr>
<td>12989</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>12990</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>12993</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>12995</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>12996</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>12997</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>12998</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>12999</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>13000</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>13001</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>13002</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>13003</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>13004</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>13005</td>
<td>Salmonella typhimurium</td>
<td>Salmonella typhimurium</td>
<td>Positive</td>
</tr>
<tr>
<td>13006</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>13007</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>13008</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>13009</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>13010</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>13011</td>
<td>Salmonella typhimurium</td>
<td>Salmonella typhimurium</td>
<td>Positive</td>
</tr>
<tr>
<td>13012</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>13013</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>13014</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>13015</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>13016</td>
<td>Salmonella kentucky</td>
<td>Salmonella kentucky</td>
<td>Positive</td>
</tr>
<tr>
<td>13017</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>13018</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>13019</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>13020</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>13021</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>13022</td>
<td>Salmonella heidelberg</td>
<td>Salmonella heidelberg</td>
<td>Positive</td>
</tr>
<tr>
<td>13035</td>
<td>Salmonella enterica</td>
<td>Salmonella enterica</td>
<td>Positive</td>
</tr>
<tr>
<td>13036</td>
<td>Salmonella enterica</td>
<td>Salmonella enterica</td>
<td>Positive</td>
</tr>
<tr>
<td>13037</td>
<td>Salmonella species</td>
<td>Salmonella species</td>
<td>Positive</td>
</tr>
<tr>
<td>13056</td>
<td>Salmonella senftenberg</td>
<td>Salmonella senftenberg</td>
<td>Positive</td>
</tr>
<tr>
<td>13057</td>
<td>Salmonella senftenberg</td>
<td>Salmonella senftenberg</td>
<td>Positive</td>
</tr>
<tr>
<td>13058</td>
<td>Salmonella senftenberg</td>
<td>Salmonella senftenberg</td>
<td>Positive</td>
</tr>
<tr>
<td>13059</td>
<td>Salmonella senftenberg</td>
<td>Salmonella senftenberg</td>
<td>Positive</td>
</tr>
<tr>
<td>Sample ID</td>
<td>Species</td>
<td>Strain</td>
<td>Result</td>
</tr>
<tr>
<td>-----------</td>
<td>--------------------------</td>
<td>-------------------------</td>
<td>--------</td>
</tr>
<tr>
<td>13060</td>
<td>Salmonella senftenberg</td>
<td>Salmonella senftenberg</td>
<td>Positive</td>
</tr>
<tr>
<td>13061</td>
<td>Salmonella tennessee</td>
<td>Salmonella tennessee</td>
<td>Positive</td>
</tr>
<tr>
<td>13062</td>
<td>Salmonella tennessee</td>
<td>Salmonella tennessee</td>
<td>Positive</td>
</tr>
<tr>
<td>13063</td>
<td>Salmonella tennessee</td>
<td>Salmonella tennessee</td>
<td>Positive</td>
</tr>
<tr>
<td>13064</td>
<td>Salmonella tennessee</td>
<td>Salmonella tennessee</td>
<td>Positive</td>
</tr>
<tr>
<td>13065</td>
<td>Salmonella tennessee</td>
<td>Salmonella tennessee</td>
<td>Positive</td>
</tr>
<tr>
<td>13066</td>
<td>Salmonella tennessee</td>
<td>Salmonella tennessee</td>
<td>Positive</td>
</tr>
<tr>
<td>13067</td>
<td>Salmonella havana</td>
<td>Salmonella havana</td>
<td>Positive</td>
</tr>
<tr>
<td>13068</td>
<td>Salmonella lexiington</td>
<td>Salmonella lexiington</td>
<td>Positive</td>
</tr>
<tr>
<td>13069</td>
<td>Salmonella mbandaka</td>
<td>Salmonella mbandaka</td>
<td>Positive</td>
</tr>
<tr>
<td>13070</td>
<td>Salmonella species</td>
<td>Salmonella species</td>
<td>Positive</td>
</tr>
<tr>
<td>13075</td>
<td>Salmonella species</td>
<td>Salmonella cubana</td>
<td>Positive</td>
</tr>
<tr>
<td>13079</td>
<td>Salmonella newport</td>
<td>Salmonella newport</td>
<td>Positive</td>
</tr>
<tr>
<td>13080</td>
<td>Salmonella saintpaul</td>
<td>Salmonella saintpaul</td>
<td>Positive</td>
</tr>
<tr>
<td>13081</td>
<td>Salmonella virchow</td>
<td>Salmonella virchow</td>
<td>Positive</td>
</tr>
<tr>
<td>S-1</td>
<td>Salmonella</td>
<td>Newport</td>
<td>Positive</td>
</tr>
<tr>
<td>S-4</td>
<td>Salmonella</td>
<td>Typhimurium</td>
<td>Positive</td>
</tr>
<tr>
<td>S-45</td>
<td>Salmonella</td>
<td>V 48:i:-</td>
<td>Positive</td>
</tr>
<tr>
<td>S-46</td>
<td>Salmonella</td>
<td>V 40:z35:&lt;&gt;</td>
<td>Positive</td>
</tr>
<tr>
<td>S-47</td>
<td>Salmonella</td>
<td>V 44:z39:&lt;&gt;</td>
<td>Positive</td>
</tr>
<tr>
<td>S-48</td>
<td>Salmonella</td>
<td>V 60:z41:-</td>
<td>Positive</td>
</tr>
<tr>
<td>S-49</td>
<td>Salmonella</td>
<td>V 66:z41:&lt;&gt;</td>
<td>Positive</td>
</tr>
<tr>
<td>S-5</td>
<td>Salmonella</td>
<td>Typhi</td>
<td>Positive</td>
</tr>
<tr>
<td>S-50</td>
<td>Salmonella</td>
<td>V 48:z35:&lt;&gt;</td>
<td>Positive</td>
</tr>
<tr>
<td>S-51</td>
<td>Salmonella</td>
<td>VI 6,14,25:z10:i,(2),7</td>
<td>Positive</td>
</tr>
<tr>
<td>S-54</td>
<td>Salmonella</td>
<td>VI 11:i,7</td>
<td>Positive</td>
</tr>
<tr>
<td>S-54</td>
<td>Salmonella</td>
<td>VI 11:1:a,l,5</td>
<td>Positive</td>
</tr>
<tr>
<td>S-55</td>
<td>Salmonella</td>
<td>VI 6,14,25:a:e,n,x</td>
<td>Positive</td>
</tr>
<tr>
<td>S-56</td>
<td>Salmonella</td>
<td>Typhimurium / DTI 04b</td>
<td>Positive</td>
</tr>
<tr>
<td>S-57</td>
<td>Salmonella</td>
<td>Typhimurium</td>
<td>Positive</td>
</tr>
<tr>
<td>S-58</td>
<td>Salmonella</td>
<td>Typhimurium</td>
<td>Positive</td>
</tr>
<tr>
<td>S-59</td>
<td>Salmonella</td>
<td>Typhimurium</td>
<td>Positive</td>
</tr>
<tr>
<td>S-60</td>
<td>Salmonella</td>
<td>Typhimurium</td>
<td>Positive</td>
</tr>
<tr>
<td>S-61</td>
<td>Salmonella</td>
<td>Typhimurium</td>
<td>Positive</td>
</tr>
<tr>
<td>S-62</td>
<td>Salmonella</td>
<td>Typhimurium</td>
<td>Positive</td>
</tr>
<tr>
<td>S-63</td>
<td>Salmonella</td>
<td>Typhimurium</td>
<td>Positive</td>
</tr>
<tr>
<td>S-64</td>
<td>Salmonella</td>
<td>Typhimurium</td>
<td>Positive</td>
</tr>
<tr>
<td>S-65</td>
<td>Salmonella</td>
<td>Typhimurium</td>
<td>Positive</td>
</tr>
<tr>
<td>S-66</td>
<td>Salmonella</td>
<td>Typhimurium</td>
<td>Positive</td>
</tr>
<tr>
<td>S-67</td>
<td>Salmonella</td>
<td>Typhimurium</td>
<td>Positive</td>
</tr>
<tr>
<td>S-68</td>
<td>Salmonella</td>
<td>Typhimurium</td>
<td>Positive</td>
</tr>
<tr>
<td>S-69</td>
<td>Salmonella</td>
<td>Typhimurium</td>
<td>Positive</td>
</tr>
<tr>
<td>S-70</td>
<td>Salmonella</td>
<td>Typhimurium / DTI 04</td>
<td>Positive</td>
</tr>
<tr>
<td>S-71</td>
<td>Salmonella</td>
<td>Typhimurium / DTI 04</td>
<td>Positive</td>
</tr>
<tr>
<td>S-72</td>
<td>Salmonella</td>
<td>Typhimurium / DTI 04</td>
<td>Positive</td>
</tr>
<tr>
<td>S-8</td>
<td>Salmonella</td>
<td>Virchow</td>
<td>Positive</td>
</tr>
<tr>
<td>S-82</td>
<td>Salmonella</td>
<td>Saphra</td>
<td>Positive</td>
</tr>
<tr>
<td>DuPont Qualicon ID#</td>
<td>ID Species</td>
<td>BAX® System Result</td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
<td>-----------------------------</td>
<td>--------------------</td>
<td></td>
</tr>
<tr>
<td>DD2901</td>
<td>Bacillus cereus</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD2558</td>
<td>Citrobacter freundii</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD383</td>
<td>Citrobacter freundii</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD1725</td>
<td>E.coli O125:H19</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD2614</td>
<td>Edwardsiella tarda</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD11348</td>
<td>Enterobacter sakazakii</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD3981</td>
<td>Enterococcus faecalis</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD846</td>
<td>Escherichia blattae</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD641</td>
<td>Escherichia coli</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD640</td>
<td>Escherichia coli</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td></td>
<td>O157:H7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DD847</td>
<td>Escherichia ferguson</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD6719</td>
<td>Escherichia hermanii</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD849</td>
<td>Escherichia intermedia</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD850</td>
<td>Escherichia vulneris</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD6121</td>
<td>Gram negative rod</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD2389</td>
<td>Hafnia alvei</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD5588</td>
<td>Hafnia alvei</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD6523</td>
<td>Klebsiella oxytoca</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD658</td>
<td>Klebsiella oxytoca</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD657</td>
<td>Klebsiella ozaenae</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD373</td>
<td>Klebsiella pneumoniae</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD7344</td>
<td>Lactobacillus acidophilus</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD687</td>
<td>Lactobacillus carnis</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD922</td>
<td>Listeria innocua</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD1152</td>
<td>Listeria monocytogenes</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD13142</td>
<td>Morganella morgani</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD3064</td>
<td>Morganella morgani</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD374</td>
<td>Proteus mirabilis</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD13147</td>
<td>Providencia rettgeri</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD13148</td>
<td>Pseudomonas aeruginosa</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD3982</td>
<td>Pseudomonas</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Species</td>
<td>Result</td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>--------------------------</td>
<td>------------</td>
<td></td>
</tr>
<tr>
<td>DD569</td>
<td>Pseudomonas aeruginosa</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD661</td>
<td>Pseudomonas fluorescens</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD577</td>
<td>Pseudomonas stutzeri</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD21 66</td>
<td>Salmonella abaeetetuba</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD301 7</td>
<td>Salmonella dublin</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD301 9</td>
<td>Salmonella dublin</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD1 777</td>
<td>Salmonella enterica</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD241 6</td>
<td>Serratia liquefaciens</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD241 7</td>
<td>Serratia liquefaciens</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD1 081</td>
<td>Shigella boydii</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD6832</td>
<td>Shigella sonnei</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD41 60</td>
<td>Staphylococcus aureus</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD61 0</td>
<td>Staphylococcus aureus</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD3998</td>
<td>Streptococcus equi</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD7083</td>
<td>Unknown</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>TD31 36</td>
<td>Vibrio cholera</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>DD1 3249</td>
<td>Vibrio parahemolyticus</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>TD31 22</td>
<td>Vibrio vulnificus</td>
<td>Negative</td>
<td></td>
</tr>
</tbody>
</table>
CLAIMS

What is claimed is:

1. A method for detecting the presence of *Salmonella* in a sample, said sample comprising nucleic acids, said method comprising
   (a) providing a reaction mixture comprising at least one primer and probe, wherein said primer is at least 11 nucleotides in length and said probe is at least 14 nucleotides in length, and wherein
   (i) said primer comprises a nucleic acid sequence capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO: 1 or a sequence complementary thereto, and said probe comprises a nucleic acid sequence capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO: 2 or a sequence complementary thereto; or
   (ii) said primer comprises a nucleic acid sequence capable of selectively hybridizing under stringent conditions to the nucleic acid sequence of SEQ ID NO: 3 or a sequence complementary thereto, and said probe comprises a nucleic acid sequence capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO: 4 or a sequence complementary thereto; and
   (b) performing PCR amplification of said nucleic acids of said sample using the reaction mixture of step (a); and
   (c) detecting the amplification of step (b).

2. The method of claim 1, wherein said reaction mixture comprises both the primer and probe of (i) and the primer and probe of (ii).
3. The method of claim 1, wherein said primer and probe each possess a 3' terminus and a 5' terminus, and wherein the 3' terminus of said probe is directly or indirectly attached to the 5' terminus of the primer, thereby forming a primer-probe complex.

4. The method of claim 3, wherein said primer-probe complex comprises a detectable label.

5. The method of claim 1 or 3, wherein said primer capable of selectively hybridizing to SEQ ID NO: 1 comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 40-54, and wherein said probe capable of selectively hybridizing to SEQ ID NO: 2 comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 6-39.

6. The method of claim 1 or 3, wherein said primer capable of selectively hybridizing to SEQ ID NO: 3 comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 95-111, and wherein said probe capable of selectively hybridizing to SEQ ID NO: 4 comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 55-94.

7. The method of claim 3, wherein said primer-probe complex further comprises a 5' Stem Sequence and a 3' Stem Sequence, wherein the 3' terminus of the 5' Stem Sequence is directly or indirectly attached to the 5' terminus of the probe, wherein the 5' terminus of the 3' Stem Sequence is directly or indirectly attached to the 3' terminus of the probe, and wherein the 3' terminus of the 3' Stem Sequence is directly or indirectly attached of the 5' terminus of the primer.

8. The method of claim 7, wherein said 5' Stem Sequence comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 112-119, and wherein said 3' Stem Sequence comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 120-130.
9. The method of claim 1 or 3, wherein said reaction mixture further comprises a quencher oligonucleotide capable of selectively hybridizing under stringent conditions to the probe.

10. An isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NOs: 6-111.

11. An isolated polynucleotide, wherein said polynucleotide comprises a primer-probe complex, wherein said primer-probe complex comprises a nucleic acid primer portion and a nucleic acid probe portion, wherein said primer portion is at least 11 nucleotides in length and said probe portion is at least 14 nucleotides in length, wherein the 3' terminus of said probe portion is directly or indirectly attached to the 5' terminus of said primer portion, and wherein

(i) said primer portion comprises a nucleic acid sequence capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO: 1 or a sequence complementary thereto, and said probe portion comprises a nucleic acid sequence capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO: 2 or a sequence complementary thereto; or

(ii) said primer portion comprises a nucleic acid sequence capable of selectively hybridizing under stringent conditions to the nucleic acid sequence of SEQ ID NO: 3 or a sequence complementary thereto, and said probe portion comprises a nucleic acid sequence capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO: 4 or a sequence complementary thereto.

12. The isolated polynucleotide of claim 11, wherein said primer portion comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 40-54, and wherein said probe portion comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 6-39.
13. The isolated polynucleotide of claim 11, wherein said primer portion comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 95-111, and wherein said probe portion comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 55-94.

14. The isolated polynucleotide of claim 11, wherein said primer-probe complex further comprises a 5' Stem Sequence and a 3' Stem Sequence, wherein the 3' terminus of the 5' Stem Sequence is directly or indirectly attached to the 5' terminus of the probe portion, wherein the 5' terminus of the 3' Stem Sequence is directly or indirectly attached to the 3' terminus of the probe portion, and wherein the 3' terminus of the 3' Stem Sequence is directly or indirectly attached of the 5' terminus of the primer portion.

15. The isolated polynucleotide of claim 14, wherein said 5' Stem Sequence comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 112-119, and wherein said 3' Stem Sequence comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 120-130.

16. A kit for detection of *Salmonella* in a sample, comprising an isolated polynucleotide of claim 11.

17. A reagent tablet comprising an isolated polynucleotide of claim 11.
<table>
<thead>
<tr>
<th>Primer-Probe Complex</th>
<th>5' Modification</th>
<th>5' Stem SEQ ID</th>
<th>5' Stem Sequence</th>
<th>Probe (Loop) SEQ ID</th>
<th>Probe (Loop) Sequence</th>
<th>3' Stem SEQ ID</th>
<th>3' Stem Sequence</th>
<th>Internal Modification</th>
<th>Primer SEQ ID</th>
<th>Primer Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primer-Probe Complex</td>
<td>5' Modification</td>
<td>5' Stem SEQ ID</td>
<td>5' Stem Sequence</td>
<td>Probe (Loop) SEQ ID</td>
<td>Probe (Loop) Sequence</td>
<td>3' Stem SEQ ID</td>
<td>3' Stem Sequence</td>
<td>Internal Modification</td>
<td>Primer SEQ ID</td>
<td>Primer Sequence</td>
</tr>
<tr>
<td>----------------------</td>
<td>-----------------</td>
<td>---------------</td>
<td>------------------</td>
<td>--------------------</td>
<td>----------------------</td>
<td>---------------</td>
<td>-----------------</td>
<td>----------------------</td>
<td>--------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>S35TEX3a</td>
<td>Cy5</td>
<td>114</td>
<td>AGGGCCCC</td>
<td>29</td>
<td>CCGGCCTGGTGGTCGGGCA</td>
<td>124</td>
<td>GGGGCCCT</td>
<td>[BHQ2][HE6]</td>
<td>47</td>
<td>TACCGGGACGCTTT</td>
</tr>
<tr>
<td></td>
<td>Texas Red</td>
<td>114</td>
<td>AGGGCCCC</td>
<td>29</td>
<td>CCGGCCTGGTGGTCGGGCA</td>
<td>124</td>
<td>GGGGCCCT</td>
<td>[BHQ2][SP-18]</td>
<td>47</td>
<td>ATCGCGGTTAAC</td>
</tr>
<tr>
<td>S761aC610-4d</td>
<td>Cal Fluor Red 610</td>
<td>116</td>
<td>ACGGCGGC</td>
<td>90</td>
<td>CGCGTCAATGACCCTGAGCGACGTGAAA</td>
<td>127</td>
<td>GCGGCGGT</td>
<td>[BHQ2][SP-18]</td>
<td>111</td>
<td>CTTTACCCGCTTCCAG</td>
</tr>
<tr>
<td>S761bC610-4g</td>
<td>Cal Fluor Red 610</td>
<td>116</td>
<td>ACGGCGGC</td>
<td>93</td>
<td>CGCGCCAATGACCATAAGCGACGTGAAA</td>
<td>127</td>
<td>GCGGCGGT</td>
<td>[BHQ2][SP-18]</td>
<td>110</td>
<td>GTGGGCTCGGA</td>
</tr>
<tr>
<td>S761bC610-5g</td>
<td>Cal Fluor Red 610</td>
<td>117</td>
<td>ACGGACCGG</td>
<td>93</td>
<td>CGCGCCAATGACCATAAGCGACGTGAAA</td>
<td>128</td>
<td>GCGGTCGGT</td>
<td>[BHQ2][SP-18]</td>
<td>110</td>
<td>TTTTACCCGCTTCCAG</td>
</tr>
<tr>
<td>S761C610-3</td>
<td>Cal Fluor Red 610</td>
<td>114</td>
<td>AGGGCCCG</td>
<td>62</td>
<td>CCTAGCCGACGCGGAAAAAT</td>
<td>124</td>
<td>GGGGCCCT</td>
<td>[BHQ2][SP-18]</td>
<td>102</td>
<td>CTTTACCCGCTTCCAG</td>
</tr>
<tr>
<td>S761C610-3a</td>
<td>Cal Fluor Red 610</td>
<td>114</td>
<td>AGGGCCCG</td>
<td>80</td>
<td>CGCGCCAATGATCCTAGCGACGTGAA</td>
<td>124</td>
<td>GGGGCCCT</td>
<td>[BHQ2][SP-18]</td>
<td>102</td>
<td>CTTTACCCGCTTCCAG</td>
</tr>
<tr>
<td>S761C610-3b</td>
<td>Cal Fluor Red 610</td>
<td>114</td>
<td>AGGGCCCG</td>
<td>88</td>
<td>GCCGTAAGCCGCGCAATGAGCTCTAGGGCAGGAAAAA</td>
<td>124</td>
<td>GGGGCCCT</td>
<td>[BHQ2][SP-18]</td>
<td>102</td>
<td>CTTTACCCGCTTCCAG</td>
</tr>
<tr>
<td>S761C610-3c</td>
<td>Cal Fluor Red 610</td>
<td>114</td>
<td>AGGGCCCG</td>
<td>89</td>
<td>GCCAATGATCTCTAAGCGAGCGGAAAAA</td>
<td>124</td>
<td>GGGGCCCT</td>
<td>[BHQ2][SP-18]</td>
<td>102</td>
<td>CTTTACCCGCTTCCAG</td>
</tr>
<tr>
<td>S761C610-4c</td>
<td>Cal Fluor Red 610</td>
<td>115</td>
<td>AGGCAGCGG</td>
<td>89</td>
<td>GCCAATGATCTCTAAGCGAGCGGAAAAA</td>
<td>126</td>
<td>GGGGCCCT</td>
<td>[BHQ2][SP-18]</td>
<td>102</td>
<td>CTTTACCCGCTTCCAG</td>
</tr>
<tr>
<td>S761C610-4d</td>
<td>Cal Fluor Red 610</td>
<td>116</td>
<td>AGGCGCGG</td>
<td>90</td>
<td>CGCGTCGAATGACCTGAGCGACGTGAAA</td>
<td>127</td>
<td>GCGGCGGT</td>
<td>[BHQ2][SP-18]</td>
<td>102</td>
<td>CTTTACCCGCTTCCAG</td>
</tr>
<tr>
<td>S761C610-4e</td>
<td>Cal Fluor Red 610</td>
<td>116</td>
<td>AGGCGCGG</td>
<td>91</td>
<td>CCGTTCGACGGTGGAATGACGTGAGGAAA</td>
<td>127</td>
<td>GCGGCGGT</td>
<td>[BHQ2][SP-18]</td>
<td>102</td>
<td>CTTTACCCGCTTCCAG</td>
</tr>
</tbody>
</table>

**FIG. 1B**
<table>
<thead>
<tr>
<th>Primer-Probe Complex</th>
<th>5’ Modification</th>
<th>5’ Stem SEQ ID</th>
<th>5’ Stem Sequence</th>
<th>Probe (Loop) SEQ ID</th>
<th>Probe (Loop) Sequence</th>
<th>3’ Stem SEQ ID</th>
<th>3’ Stem Sequence</th>
<th>Internal Modification</th>
<th>Primer SEQ ID</th>
<th>Primer Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>S761C610-4f</td>
<td>Cal Fluor Red 610</td>
<td>116</td>
<td>ACGGCCGC</td>
<td>92</td>
<td>GTCAATGACCTGAGCGACGTGAAA</td>
<td>127</td>
<td>GCGGCCGCT</td>
<td>[BHQ2][SP-18]</td>
<td>102</td>
<td>CTTTACCCGCTTCACGTTGGCCCTGAA</td>
</tr>
<tr>
<td>S761C610-4g</td>
<td>Cal Fluor Red 610</td>
<td>116</td>
<td>ACGGCCGC</td>
<td>93</td>
<td>CGCGCCAATGACCATAGACCGACGTGAA</td>
<td>127</td>
<td>GCGGCCGCT</td>
<td>[BHQ2][SP-18]</td>
<td>102</td>
<td>CTTTACCCGCTTCACGTTGGCCCTGAA</td>
</tr>
<tr>
<td>S761C610-5f</td>
<td>Cal Fluor Red 610</td>
<td>117</td>
<td>ACGGACCGC</td>
<td>92</td>
<td>GTCAATGACCTGAGCGACGTGAA</td>
<td>128</td>
<td>GCGGCCTCCTGAA</td>
<td>[BHQ2][SP-18]</td>
<td>102</td>
<td>CTTTACCCGCTTCACGTTGGCCCTGAA</td>
</tr>
<tr>
<td>S761C610-5g</td>
<td>Cal Fluor Red 610</td>
<td>117</td>
<td>ACGGACCGC</td>
<td>93</td>
<td>CGCGCCAATGACCATAGACCGACGTGAA</td>
<td>128</td>
<td>GCGGCCTCCTGAA</td>
<td>[BHQ2][SP-18]</td>
<td>102</td>
<td>CTTTACCCGCTTCACGTTGGCCCTGAA</td>
</tr>
<tr>
<td>SB35C610</td>
<td>Cal Fluor Red 610</td>
<td>29</td>
<td>CGGGCGCTGGTGTCGGCA</td>
<td></td>
<td></td>
<td>[SP-18]</td>
<td></td>
<td></td>
<td>47</td>
<td>TAGCCCGGACGCTTTAATCCGCGTTAAC</td>
</tr>
<tr>
<td>SB761C610</td>
<td>Cal Fluor Red 610</td>
<td>90</td>
<td>CGGTCAATGACCTGAGCGACGTGAA</td>
<td></td>
<td></td>
<td>[SP-18]</td>
<td></td>
<td></td>
<td>102</td>
<td>CTTTACCCGCTTCACGTTGGCCCTGAA</td>
</tr>
<tr>
<td>SB761C610-g</td>
<td>Cal Fluor Red 610</td>
<td>93</td>
<td>CGCGCCAATGACCATAGACCGACGTGAA</td>
<td></td>
<td></td>
<td>[SP-18]</td>
<td></td>
<td></td>
<td>102</td>
<td>CTTTACCCGCTTCACGTTGGCCCTGAA</td>
</tr>
<tr>
<td>S4219EC5603b</td>
<td>Cal Fluor Orange 560</td>
<td>132</td>
<td>AGGCCGCC</td>
<td>133</td>
<td>CAGACGACCGCCGCCGCT</td>
<td>134</td>
<td>GCCGCCT</td>
<td>[BHQ1][SP-18]</td>
<td>135</td>
<td>GACGATGATATATTAAAGCACACTCTATGCTGTG</td>
</tr>
</tbody>
</table>

**FIG. 1C**
**INTERNATIONAL SEARCH REPORT**

**International application No**
PCT/US2012/063799

**A. CLASSIFICATION OF SUBJECT MATTER**

INV. C12Q1/68

ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, BIOSIS, Sequence Search, EMBASE

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>WO 95/33854 A1 (DU PONT DE NEMOURS &amp; CO E) 14 December 1995 (1995-12-14) sequences 1Q, 15, 19</td>
<td>10</td>
</tr>
</tbody>
</table>

* Special categories of cited documents:

**A** document defining the general state of the art which is not considered to be of particular relevance

**E** earlier application or patent but published on or after the international filing date

**L** document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

**O** document referring to an oral disclosure, use, exhibition or other means

**P** document published prior to the international filing date but later than the priority date claimed

**X** later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

**Y** document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

**Y** document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

**X** document member of the same patent family

Date of the actual completion of the international search

4 January 2013

Date of mailing of the international search report

17/01/2013

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk
Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016

Authorized officer

Santagati, Fabi

Form PCT/ISA/210 (second sheet) (April 2009)
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>REYNISSON E ET AL: &quot;Evaluation of probe chemistries and platforms to improve the detection limit of real-time PCR&quot;, JOURNAL OF MICROBIOLOGICAL METHODS, ELSEVIER, AMSTERDAM, NL, vol. 66, no. 2, 1 August 2006 (2006-08-01), pages 206-216, XP027926944, ISSN: 0167-7012 [retrieved on 2006-08-01] page 208, left-hand column, line 3 - line 4</td>
<td>11-17</td>
</tr>
</tbody>
</table>
1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
   a. (means)
      □ on paper
      □ in electronic form
   b. (time)
      □ in the international application as filed
      □ together with the international application in electronic form
      □ subsequently to this Authority for the purpose of search

2. □ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:
## INTERNATIONAL SEARCH REPORT

**Information on patent family members**

<table>
<thead>
<tr>
<th>Patent document cited in search report</th>
<th>Publication date</th>
<th>Patent family member(s)</th>
<th>Publication date</th>
</tr>
</thead>
<tbody>
<tr>
<td>WO 9533854 A1</td>
<td>14-12-1995</td>
<td>AT 224453 T</td>
<td>15-10-2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 2189546 A</td>
<td>14-12-1995</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DE 69528263 D1</td>
<td>24-10-2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DE 69528263 T2</td>
<td>07-08-2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 0764217 A</td>
<td>26-03-1997</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP H09506002 A</td>
<td>17-06-1997</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 5656740 A</td>
<td>12-08-1997</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 5660981 A</td>
<td>26-08-1997</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 9533854 A</td>
<td>14-12-1995</td>
</tr>
</tbody>
</table>

| | | AU 5736194 A | 04-07-1994 |
| | | CA 2151036 A | 23-06-1994 |
| | | DE 69308162 D1 | 27-03-1997 |
| | | DE 69308162 T2 | 26-06-1997 |
| | | DK 675969 T3 | 28-04-1997 |
| | | EP 0675969 A | 11-10-1995 |
| | | ES 2097633 T3 | 01-04-1997 |
| | | GR 3023325 T3 | 29-08-1997 |
| | | JP H08504332 A | 14-05-1996 |
| | | US 5340728 A | 23-08-1994 |
| | | WO 9413832 A1 | 23-06-1994 |

| | | CA 2522689 A1 | 28-10-2004 |
| | | EP 1616029 A2 | 18-01-2006 |
| | | US 2007134659 A1 | 14-06-2007 |
| | | WO 2004092408 A2 | 28-10-2004 |