



- (51) International Patent Classification:
C12Q 1/68 (2006.01)
- (21) International Application Number:
PCT/EP2016/066209
- (22) International Filing Date:
7 July 2016 (07.07.2016)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
15306154.4 10 July 2015 (10.07.2015) EP
- (71) Applicants: INSTITUT PASTEUR [FR/FR]; 25-28, rue du Docteur Roux, 75724 Paris Cedex 15 (FR). UNIVERSITE PARIS DESCARTES [FR/FR]; 12, rue de l'Ecole de Médecine, 75006 Paris (FR). UNIVERSITE PARIS DIDEROT - PARIS 7 [FR/FR]; 5, rue Thomas Mann, 75205 Paris Cedex 13 (FR). CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE [FR/FR]; 3, rue Michel-Ange, 75794 Paris Cedex 16 (FR). ASSISTANCE PUBLIQUE - HOPITAUX DE PARIS [FR/FR]; 3, avenue Victoria, 75004 Paris (FR).
- (72) Inventors: CHENAL-FRANCISQUE, Viviane; 8, villa des Olivettes, 92220 Bagneux (FR). MAURY, Mylène; 1, boulevard Dubreuil, 91400 Orsay (FR). LAVINA, Morgane; 43, avenue Vladimir Illitch Lénine, 94110 Arcueil (FR). TOUCHON, Marie; 245, avenue d'Argenteuil, 92270 Bois-Colombes (FR). LECLERCQ, Alexandre; 17bis, avenue Jean Jaurès, 75019 Paris (FR). LECUIT, Marc; 74, boulevard Raspail, 75006 Paris (FR). BRISSE, Sylvain; 31, rue Drouet Peupion, 92140 Clamart (FR).
- (74) Agents: GUTMANN, Ernest et al.; Yves Plasseraud SAS, 3, rue Auber, 75009 Paris (FR).
- (81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
- (84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH,

[Continued on next page]

- (54) Title:
- LISTERIA MONOCYTOGENES*
- CLONOGROUPING AND ASSESSMENT OF INFECTIVITY

(57) Abstract: The application relates to *Listeria monocytogenes*, more particularly to the determination of the clone to which a *L. monocytogenes* isolate belongs. The means of the application involve primers and/or probes, more particularly multiplex primers. The means of the application are notably useful to assess the invasivity that said *L. monocytogenes* isolate might show in a human being.

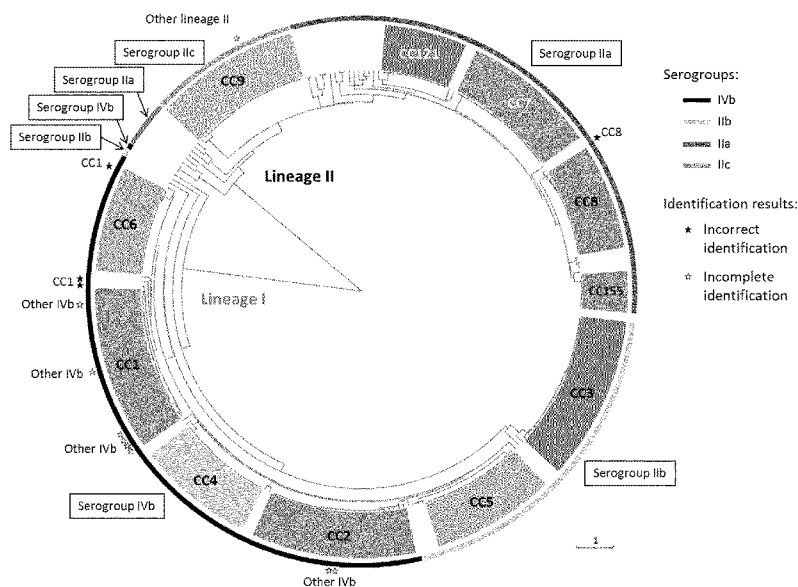


FIGURE 7



GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

TITLE

Listeria monocytogenes clonogrouping and assessment of infectivity

TECHNICAL FIELD

5

The application relates to *Listeria monocytogenes*, more particularly to *L. monocytogenes* clonogrouping, *i.e.*, to the determination of the clone to which a *L. monocytogenes* isolate belongs. The means of the application involve primers and/or probes, more particularly primers suitable for use in multiplex amplification. The means of the application are notably useful to assess the infectivity that a *L. monocytogenes* isolate might show in a human being.

10

BACKGROUND

Listeria monocytogenes (*L. monocytogenes*) is a major human foodborne pathogen and causes infections in animals as well. It is responsible for 360 human invasive infection cases in average per year in France. The Centers for Disease Control and prevention (CDC) in the U.S.A. estimate that approximately 1,600 illnesses and 260 deaths due to listeriosis occur annually in the U.S.A., and similar numbers of listeriosis are estimated to occur in Europe. *L. monocytogenes* infects mostly the elderly, the immunocompromised individuals and pregnant women. Besides gastroenteritis, three types of invasive infections can be caused by *L. monocytogenes*: bacteremia, central nervous system infection and materno-fetal infections that often lead to abortion. These invasive infections are characterized by a high fatality rate (estimated between 20 to 30 % of cases) and infections always require hospitalization.

20

Food industry is strongly concerned by contamination with *L. monocytogenes* and regulations are in place in Western countries on the acceptable levels of food contamination, which implies intense monitoring, including quantification of levels of *L. monocytogenes* contamination of food products and strain typing activities in food industry, national reference centers and food safety reference laboratories. In the food chain, food business operators and competent authorities manage the *L. monocytogenes* hazard by its detection and/or enumeration in samples of food chain and/or food environment, followed by its characterization at the serotype or genoserotype level. When contamination is above a defined level (assessed for example by the number of colony forming units per gram of food product), the products have to be very rapidly recalled and withdrawn from the market, the production plant has to be treated for elimination of the bacteria, and consumers have to be immediately alerted and informed.

30

A *L. monocytogenes* strain can be classified in lineages, serogroups, serotypes, clonal complexes (CCs, which are further referred as clones), or in PFGE types that are determined by Pulsed-Field

35

Gel Electrophoresis (PFGE) using one or two restriction enzymes (generally *AscI* and/or *ApaI*) (*cf. e.g., Brosch et al. 1994, Graves et al. 2001*).

Lineage is usually determined by sequence-based studies (*Ward et al. 2008*). There are four lineages, *i.e.*, lineage I, lineage II, lineage III and lineage IV.

5 Serogroup (also called PCR serogroup or genoserogroup in the literature) is determined by the reference multiplex PCR method of Doumith *et al.* 2004, which involves the PCR analysis of particular target regions in four marker genes and one control gene. The four marker genes are *lmo1118* (906 bp fragment), *lmo0737* (691 bp fragment), *ORF2110* (597 bp fragment) and *ORF2819* (471 bp fragment), within which the targeted regions are of 906 bp, 691 bp, 597 bp and
10 471 bp, respectively. The control gene is *prs*, within which the targeted region is of 370 bp. There are four serogroups, *i.e.*, serogroup IVb (including mostly serotype 4b strains, but also strains of serotypes 4d and 4e), serogroup I Ib (including mostly serotype 1/2b strains, but also strains of serotypes 3b and 7), serogroup IIa (including mostly serotype 1/2a strains, but also strains of serotype 3a) and serogroup IIc (including mostly serotype 1/2c strains, but also strains of serotype
15 3c).

Serotype is determined by serotyping of somatic and flagellar antigens (*cf. e.g., Seeliger and Hohne 1979*). Examples of serotypes notably include serotypes 4b, 1/2b, 1/2a and 1/2c.

Clone identity is determined by the Multilocus Sequence Typing (MLST) method of Ragon *et al.* 2008, which involves the PCR and sequencing analysis of internal regions of seven housekeeping
20 genes, *i.e.*, *acbZ* (ABC transposase), *bgIA* (beta-glucosidase), *cat* (catalase), *dapE* (succinyl diaminopimelate desuccinylase), *dat* (D-amino acid aminotransferase), *ldh* (lactate dehydrogenase) and *lhcA* (histidine kinase)). Examples of clones include clone CC1, clone CC2, clone CC3, clone CC4, clone CC5, clone CC6, clone CC7, clone CC8, clone CC16, clone CC9, clone CC121 and clone CC155. Worldwide distribution of these clones has been confirmed in Chenal-Francisque *et al.* in 2011.
25

Serotypes reflect the antigenic properties of lipoteichoic acids and the flagellin at the surface of bacteria. The other methods reflect the phylogenetic origin of the strains. There is a strong concordance between the different phylogenetic categories. For example,

- clones CC1, CC2, CC4 and CC6 are mainly of serotypes 4b, 4d and 4e, are of serogroup IVb and
30 of lineage I;
- clones CC3 and CC5 are mainly of serotypes 1/2b and 3b, are of serogroup I Ib and of lineage I;
- clones CC7, CC8, CC16, CC121 and CC155 are mainly of serotypes 1/2a and 3a, are of serogroup IIa and of lineage II; and
- clone CC9 is mainly of serotypes 1/2c, 1/2a and 3c, is of serogroups IIc and IIa and of lineage II.

35 The method, which food business operators and risk managers currently follow to evaluate the severity of a *L. monocytogenes* contamination, relies essentially on the epidemiological finding that serotypes 1/2a, 1/2b, 1/2c and 4b are the serotypes that are the most frequently observed in

human infections or food sources. There is currently neither stratification of infectivity levels of clones nor classification of the clones according to their frequency in human cases, their specific clinical forms or their lethality rate.

5 Infectivity (or infectiousness) corresponds to the potentiality of a pathogen of causing an invasive infection. Here, infectivity is assessed as the proportion of humans that become infected when exposed to the pathogen.

Serogrouping is relatively easy to implement but has limited discrimination, therefore providing limited resolution for epidemiological typing. Indeed, in order to ensure efficient industrial process contamination analyses and epidemiological surveillance of listeriosis, it is of first importance to
10 accurately identify the clones. However, the MLST method classically used to identify the clones is time-consuming and too expensive for a routine use in most industrial, private or public laboratories involved in *L. monocytogenes* typing.

A further impediment is that there was not sufficient typing and genome data to cover the whole diversity of *L. monocytogenes* clones, and that there was not sufficient clinical and bacteriological
15 data to identify those clones, which have the highest potentiality of causing an invasive infection in a human.

There is still a need for means allowing for easy and efficient identification of the *L. monocytogenes* clones, more particularly of those clones which have the potentiality, more particularly a high potentiality, of causing an invasive infection in a human.
20

SUMMARY OF THE APPLICATION

The means of the application allow detecting that a product contains a *L. monocytogenes* isolate, which has a high probability of having a potentiality, more particularly a high potentiality, of
25 causing an invasive infection in a human. The means of the application notably comprise methods, oligonucleotides (primers and/or probes, more particularly primers), sets of oligonucleotides (comprising primers and/or probes, more particularly primers), kits, computer program products and computer devices.

By contrast to the prior art, the inventors adopted a clone-specific approach.

30 They identified the *L. monocytogenes* clones, which are the most relevant to assess a high probability of having a potentiality of causing an invasive infection in a human. The relevant clones identified by the inventors comprise the CC1, CC6, CC2, CC4, CC8-CC16, CC5, CC3, CC7 and CC155 clones, and may further comprise the CC121 and CC9 clones. The potentiality of causing invasive infection in a human is lower for clones CC121 and CC9 than for clones CC1,
35 CC6, CC2, CC4, CC8-CC16, CC5, CC3, CC7 and CC155 clones. Therefore, *L. monocytogenes* isolates of clones CC1, CC6, CC2, CC4, CC8-CC16, CC5, CC3, CC7 and CC155 have a high probability of having a potentiality, more particularly a high potentiality for clones CC1, CC2,

CC4, CC6 and CC7, of causing an invasive infection in a human, whereas *L. monocytogenes* isolates of clones CC121 and CC9 have a low probability of having a potentiality, more particularly a high potentiality, of causing an invasive infection in a human.

5 A potentiality of a *L. monocytogenes* isolate of causing an invasive infection in a human can be considered to be a high potentiality when the isolate belongs to a clone that is significantly more associated to clinical origin than to food origin.

Conversely, a potentiality of a *L. monocytogenes* isolate of causing an invasive infection in a human can be considered to be a low potentiality when the isolate belongs to a clone that is significantly more associated to food origin than to clinical origin.

10

The inventors identified genes that are specific of each one of said relevant clones (genes of SEQ ID NOs: 1, 61-71, 2, 72-79, 3, 80-85, 4, 86-106, 5, 107-111, 6, 112-122, 7, 123-128, 8, 129-141, 9, 142-149, 10, 150-155 and 11).

15 To achieve this result, the inventors had to overcome several technical impediments, such as the absence in the prior art of sufficient typing and genome data to cover the whole diversity of *L. monocytogenes* clones, and the absence of the *L. monocytogenes* pan-genome.

Furthermore, the finding that clone specificity can be achieved with a single marker gene, *i.e.*, that no gene combination is needed to achieve clone specificity, is highly surprising in view of the prior art knowledge, *e.g.*, in view of the occurrence of horizontal gene transfer among
20 *L. monocytogenes* lineages.

The inventors further identified genes, which are specific of each of said relevant clones, and which allow clone identification by multiplex PCR (genes of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11).

25 Examples of multiplex PCR implementation are shown in Table 9 below (*cf.* example 3), which shows three distinct multiplex, *i.e.*, multiplex IVb, multiplex IIb and multiplex IIaIIc. The three multiplex can be implemented simultaneously, since they can share the same thermocycling conditions.

BRIEF DESCRIPTION OF THE FIGURES

30

Figure 1. Cumulative percentage of *L. monocytogenes* clones in clinical and food isolates.

Figures 2A (top) and 2B (bottom). Relative proportions (A) and numbers (B) of *L. monocytogenes* isolates from clinical and food sources per clone.

35 **Figure 3.** Result of the analysis of 2005-2013 *L. monocytogenes* isolates: nearly 80% of them were represented by 13 MLST clones only. Eleven of them are the most frequent clones for clinical isolates, *i.e.*, clones CC1, CC2, CC4, CC6, CC3, CC5, CC121, CC155, CC7, CC8-16 and CC9 (*cf. e.g.*, Figure 1, upper panel).

Figures 4A (top), 4B (middle) and 4C (bottom). Result of the analysis of 2005-2013 *L. monocytogenes* isolates: distribution of these isolates according the source from which they have been isolated (Figure 4A: food + clinical sources; Figure 4B: clinical sources; Figure 4C: food source). Clones CC1, CC2, CC4 and CC6 represented almost half of the clinical isolates, and clones CC9 and CC121 represent more than 40% of the food isolates.

Figure 5. Minimum spanning tree showing that the (more than one hundred) *L. monocytogenes* strains analyzed in genomic comparisons are representative of a large diversity of *L. monocytogenes* strains.

Figures 6A (top), 6B (middle) and 6C (bottom). PCR amplification patterns obtained for representatives of eleven target clones. Agarose gel electrophoresis of PCR products generated by (A) IVb clonogrouping multiplex PCR, (B) IIb clonogrouping multiplex PCR and (C) IIaIIc clonogrouping multiplex PCR. Lanes 2 to 12 correspond to *L. monocytogenes* strains of clones CC1 (CLIP2013/00068), CC2 (CLIP2012/00799), CC4 (CLIP2011/00181), CC6 (CLIP2010/01520), CC5 (CLIP2011/00140), CC3 (CLIP2011/00787), CC121 (CLIP2012/00189), CC155 (2010/01410), CC7 (CLIP2012/01275), CC8 (CLIP2012/01049) and CC9 (CLIP2010/01314), respectively. Lane 13: negative control. Lane 1 is the Smart Ladder small fragment (EUROGENTEC). Genes corresponding to the amplified fragments are indicated on the right. Molecular sizes are given (in base pairs) on the left.

Figure 7. Phylogenetic tree based on the concatenation of the seven MLST gene sequences of the 282 strains that were tested using the three multiplex PCRs of the application. Branches of lineage I are in grey and branches of lineage II are in black. Clones defined based on MLST are indicated with areas at the leaves. Circular arc areas around the tree indicate the serogroups based on the reference PCR serogrouping method (Doumith *et al.* 2004). Incomplete identifications are indicated by white stars, incorrect identifications are indicated by black stars.

DETAILED DESCRIPTION

In the application, unless specified otherwise or unless a context dictates otherwise, all the terms have their ordinary meaning in the relevant field(s).

For example, an isolate is defined as a clonal population of bacteria isolated from a specific source at a given time, *e.g.* an isolate from a patient or a food sample, and a strain refers to an isolate which has been characterized by some phenotypic or genotypic method(s). Unlike an isolate, a strain is preserved as a sample of the diversity of a bacterial specie but its original source is not necessarily known because of the potential loss of this information. In the application, the terms “isolate” and “strain” are used interchangeably.

For example, Clonal Complex (CC) identity is determined by the Multilocus Sequence Typing (MLST) method of Ragon *et al.* 2008, which involves the PCR analysis of seven housekeeping genes, *i.e.*, *acbZ* (ABC transposase), *bglA* (beta-glucosidase), *cat* (catalase), *dapE* (succinyl
 5 diaminopimelate desuccinylase), *dat* (D-amino acid transferase), *ldh* (lactate dehydrogenase) and *lhkA* (histidine kinase)). PCR conditions and primers are described in the section “Multilocus Sequence Typing” and in Table 1 of Ragon *et al.* 2008. The *L. monocytogenes* MLST data base is available from: <http://www.pasteur.fr/mlst>.

Examples of clones include clone CC1, clone CC2, clone CC3, clone CC4, clone CC5, clone CC6,
 10 clone CC7, clone CC8, clone CC16, clone CC9, clone CC121 and clone CC155. Worldwide distribution of these clones has been confirmed in Chenal-Francisque *et al.* 2011.

A *L. monocytogenes* strain belongs to a particular Clonal Complex (CC) [*e.g.*, to CC1], if the sequence type (ST) of this strain differs from at least one other member of the CC [*e.g.*, from the other members of CC1] by no more than one of said seven housekeeping genes. For example, a
 15 *L. monocytogenes* strain belongs to a particular Clonal Complex (CC) [*e.g.*, to CC1], if the sequence type (ST) of this strain differs from the other members of the CC [*e.g.*, from the other members of CC1] by only one of said seven housekeeping genes.

For example, serogroups (IVb, IIb, IIa and IIc) of *L. monocytogenes* refers to the serogroups as
 20 defined in Doumith *et al.* 2004, the content of which is herein incorporated by reference.

Briefly, Doumith *et al.* 2004 describes a multiplex PCR serogrouping method, which involves four marker genes, *i.e.*, *lmo0737*, *lmo1118*, ORF2819 and ORF2110. The primer pairs that are used to amplify target regions from said genes are shown in Table 1 of Doumith *et al.* 2004 (SEQ ID NOs: 182-183, 47-48, 184-185 and 186-187 in the appended sequence listing, respectively).

25 The multiplex PCR serogrouping method of Doumith *et al.* 2004 may further involve a control gene, *e.g.*, *prs*. The size of the target region in *prs* is of 370 bp. The primer pair for amplification of this *prs* target region may *e.g.*, the primer pair of SEQ ID NO: 188 (GCTGAAGAGATTGCGAAAGAAG) and SEQ ID NO: 189 (CAAAGAAACCTTGGATTTGCGG), or any other primer pair, which amplifies the same 370 bp
 30 target region.

The amplification reactions are performed as described in Doumith *et al.* 2004. Briefly, the amplification reactions of the multiplex PCR serogrouping method of Doumith *et al.* 2004 can be performed in a 96-well plate (ABGENE) in a final volume of 100 µl containing 2 U of Taq DNA polymerase (ROCHE, BOEHRINGER), 0.2 mM deoxynucleoside triphosphates (PERKIN-
 35 ELMER), and 50 mM Tris-HCl–10 mM KCl–50 mM (NH₄)₂SO₄–2 mM MgCl₂, pH 8.3. The primer sets can be added at the following final concentrations: 1 µM for the *lmo0737*, ORF2819, and ORF2110 primers; 1.5 µM for the *lmo1118* primers (0.2 µM for the control *prs* primers). PCR

can be performed with an initial denaturation step at 94°C for 3 min; 35 cycles of 94°C for 0.40 min, 53°C for 1.15 min, and 72°C for 1.15 min; and one final cycle of 72°C for 7 min in a thermocycler (ICYCLER; BIO-RAD LABORATORIES). Five microliters of the reaction mixture can then be mixed with 3 µl of loading buffer and separated on a 2% agarose gel in a TBE buffer
5 (90 mM TRIZMA base, 90 mM boric acid, 2 mM EDTA, pH 8.3). The PCR product can be visualized by ethidium bromide staining.

The means of the application notably allow determining or detecting that a product, more particularly a product intended for administration to a human (*e.g.*, a product intended for
10 administration to a human *via* the oral or intra-gastric route or the intra-peritoneal route, more particularly *via* the oral or intra-gastric route, such as a food or beverage intended for human consumption) is pathogenic (*i.e.*, toxic) to human. Indeed, the means of the application allow detecting that said product contains a *L. monocytogenes* isolate, which has a high probability of having a potentiality, more particularly a high potentiality, of causing an invasive infection in a
15 human. More particularly, the means of the application allow detecting that said product is highly pathogenic (*i.e.*, toxic) to human, *i.e.*, that said product contains a *L. monocytogenes* isolate, which has a high probability of having a potentiality, more particularly a high potentiality, of causing an invasive infection in a human.

The expression “*invasive infection*” is intended in accordance with its ordinary meaning in the
20 field. It notably encompasses a life-threatening infection or an infection where said *L. monocytogenes* isolate is present in a normally sterile body tissue (*e.g.*, blood), more particularly an infection where said *L. monocytogenes* isolate is present in a normally sterile body tissue (*e.g.*, blood). Life-threatening infections or infections where said *L. monocytogenes* isolate is present in a normally sterile body tissue notably include (*Listeria*-induced) diseases or
25 conditions, such as listeriosis in pregnancy, listeriosis of the Central Nervous System, bloodstream infection, brain abscess, glandular listeriosis, eye infection, hepatitis or liver disease, peritonitis or abdominal infection, lung infection, joint infection, arthritis, heart disease, bone infection, and gallbladder infection.

Said potentiality of causing an invasive infection is particularly high, where the human to whom
30 the product is intended to be administered, is an immunocompromised human, a pregnant woman, a fetus, a neonate, an infant, a child, or an elderly.

To achieve this advantageous result, the inventors had to overcome a number of prejudices and impediments.

Indeed, by contrast to the prior art and common general approach in the field, the inventors decided to follow a clone-specific approach. Achieving a clone-specific approach required to overcome several technical impediments.

5 The prior art data and material did not provide sufficient typing and genome data to cover the whole diversity of *L. monocytogenes* clones, and did not provide sufficient clinical and bacteriological data to identify those clones, which have a potentiality, more particularly a high potentiality, of causing an invasive infection in a human. Therefore, the inventors built a set of *L. monocytogenes* genomes, which covers *L. monocytogenes* diversity (cf. example 1 below; cf.
10 Figure 5). This set notably required *L. monocytogenes* strains to be newly isolated by the inventors. The resulting set built by the inventors consisted of 104 *L. monocytogenes* genomes, which were determined for strains that were representative of the diversity of 6,000 *L. monocytogenes* strains, which cover the world-wide diversity of *L. monocytogenes* clones.

The inventors collected clinical and bacteriological data for these 6,000 strains set, and searched
15 for those *L. monocytogenes* clones, which have a potentiality, more particularly a high potentiality, of causing an invasive infection in a human. The relevant clones identified by the inventors are the CC1, CC6, CC2, CC4, CC8-CC16, CC5, CC3, CC7 and CC155 clones (clones CC8 and CC16 have very similar Pulsed Field Electrophoresis (PFGE) profiles and therefore have been merged to constitute one epidemiologic group, i.e., clone CC8-CC16). The relevant clones identified by the
20 inventors may further comprise clones CC9 and CC121 which have a low potentiality of causing an invasive infection in a human. Please see example 1 below as well as Figures 1, 2A, 2B, 4A, 4B and 4C.

The identification of those *L. monocytogenes* clones, which have a potentiality, more particularly a
25 high potentiality, of causing an invasive infection in a human allowed the inventors to search whether some marker genes or some marker gene combinations could be found to specifically detect each of these relevant clones. The inventors therefore built a pan-genome of *L. monocytogenes*, based on a total of 104 genomes covering the *L. monocytogenes* clones. Please note that a pan-genome of *L. monocytogenes* strains representing this diversity of clones was not
30 available in the prior art.

The inventors identified genes that are specific of each of said relevant clones. These genes are identified in Table 2 below (cf. example 2): SEQ ID NOs: 1, 61-71, 2, 72-79, 3, 80-85, 4, 86-106, 5, 107-111, 6, 112-122, 7, 123-128, 8, 129-141, 9, 142-149, 10, 150-155 and 11. Each of these
35 genes is specific of one *L. monocytogenes* clone, i.e., of one of the CC1, CC2, CC4, CC6, CC3, CC5, CC121, CC155 CC7, CC8-CC16, and CC9 clones. The finding that clone specificity can be achieved with a single marker gene, i.e., that no gene combination is needed to achieve clone

specificity, is highly surprising in view of the prior art knowledge, *e.g.*, in view of the diversity of clones and the dynamics of gene transfer in bacterial species, including *L. monocytogenes*.

The inventors further identified control genes, which are specific of a *L. monocytogenes* lineage (lineage I or II), of a *L. monocytogenes* serogroup (serogroup IVb), or of a *L. monocytogenes* serogroup and lineage (serogroup IIb and lineage II). These control genes are identified in Table 3
5 below (*cf.* example 2): SEQ ID NOs: 12, 156-157, 13, 158-171, 14, 172-179, 15 and 180-181.

The inventors further achieved a selection of particular genes within the sets of genes they identified. Indeed, they selected genes that allowed clone identification by multiplex PCR (and
10 further identified control genes that are suitable for implementation in multiplex in said multiplex PCR clone identification). Each of the selected genes achieves clone specificity as single marker gene and allows implementation in multiplex PCR. These (clone-specific) genes are identified in Table 4 below (*cf.* example 2). The genes that allow multiplex PCR identification of clones CC1, CC6, CC2, CC4, CC8-CC16, CC5, CC121, CC3, CC7, CC155 and CC9 clones notably comprise
15 the genes of SEQ ID NOs: 1-11 (*cf.* Table 4 below; *cf.* example 2 below and the herewith enclosed sequence listing). The genes that allow multiplex PCR identification of the control genes notably comprise the genes of SEQ ID NOs: 12-15 (*cf.* Table 5 below; *cf.* example 2 below and the herewith enclosed sequence listing). Examples of regions that can be targeted within said genes and examples of primers that are suitable for multiplex PCR implementation comprise those
20 identified in Tables 6 and 7 below (*cf.* example 3: target regions and primers of SEQ ID NOs: 16-48 for the marker genes; target regions and primers of SEQ ID NOs: 49-60 for the control genes). Examples of multiplex PCR implementation are shown in Table 9 below (*cf.* example 3), which shows three distinct multiplex, *i.e.*, multiplex IVb, multiplex IIb and multiplex IIaIIc. The three multiplex can be implemented separately or sequentially. They can even be implemented
25 simultaneously in a single PCR instrument, since they may share the same thermocycling conditions.

Each of the three multiplex PCR of the application can be implemented separately, *i.e.*, on its own without implementation of the other two multiplex PCR, or can be implemented sequentially or simultaneously with one or the two other multiplex PCR of the application.

30 Furthermore, each of the three multiplex PCR of the application can be implemented sequentially in combination with a serogrouping method, such as the serogrouping method of Doumith *et al.* 2004. More particularly, a serogrouping method, such as the serogrouping method of Doumith *et al.* 2004, can be implemented to determine whether the isolate under test is of serogroup IVb, IIb, IIa or IIc; the multiplex PCR of the application that corresponds to the (IVb, IIb or IIaIIc) serogroup thus determined can then be implemented (*i.e.*: multiplex IVb of the application when
35 the serogroup is IVb, multiplex IIb of the application when the serogroup is IIb, multiplex IIaIIc of the application when the serogroup is IIa or IIc).

The performance of the means of the application was demonstrated on 282 *L. monocytogenes* strains representing the whole diversity of the (MLST) clones of *L. monocytogenes*, including strains of target clones as well as other clones. At least 95.4% of the strains could be identified accurately (*cf.* example 3; *cf.* Tables 10 and 11 below).

This result confirmed that the means of the application are suitable for implementation on *L. monocytogenes* isolates, *e.g.*, isolates from food or human samples, and that they are reliable to identify the clone, to which the isolate belongs or to assess the infectivity risk that this isolate might show in a human.

The means of the application further show a high specificity with respect to the *L. monocytogenes* species. Therefore they allow the identification of the *L. monocytogenes* species. They have the advantage that the *L. monocytogenes* species can be identified without requiring any other or further assay, such as any further biochemical assay.

Compared to the prior art MLST clonogrouping method, the means of the application shows the advantage of allowing clone identification on a large scale basis. For example, the PCR means of the application allow at least 96 samples to be processed simultaneously. Compared to MLST, the means of the invention are faster to perform, with results obtainable within *e.g.*, 4 to 6 hours or even less. Furthermore, the means of the application require less nucleic acid material in the initial sample to be analyzed, and are highly reproducible. The results obtained with the means of the application are easy to interpret, and need no highly specialized expertise.

Throughout the application, the expressions “high probability” and “low probability” are intended in accordance with their respective ordinary meanings in the field.

For example, a high probability is a probability of at least 80%, or at least 85%, or at least 90%, or at least 95%, more particularly of at least 95%.

For example, a low probability is a probability of less than 95%, or less than 90%, or less than 85%, or less than 80%, more particularly of less than 95%.

For example, a high probability is a probability of at least 80% and a low probability is a probability of less than 80%.

For example, a high probability is a probability of at least 85% and a low probability is a probability of less than 85%.

For example, a high probability is a probability of at least 90% and a low probability is a probability of less than 90%.

For example, a high probability is a probability of at least 95% and a low probability is a probability of less than 95%.

More particularly, a high probability of having, being, belonging, causing (or any similar verb) a certain feature encompasses the meaning of having, being, belonging, causing (or any similar verb) said feature.

Similarly, a low probability of having, being, belonging, causing (or any similar verb) a certain
5 feature encompasses the meaning of not having, not being, not belonging, not causing (or any similar verb) said feature.

The means of the application notably comprise methods, oligonucleotides (primers and/or probes, more particularly primers), sets of oligonucleotides (comprising primers and/or probes, more
10 particularly primers), kits, computer program products and computer devices.

The methods of the application comprise:

- an *in vitro* method for determining that an isolate of *Listeria monocytogenes* has a high probability of belonging to clone CC1, to clone CC2, to clone CC4, to clone CC6, to clone CC7, to
15 clone CC3, to clone CC5, to clone CC8 or CC16, or to clone CC155 of *L. monocytogenes* (wherein a high probability of belonging to one of these clones is indicative that said *L. monocytogenes* isolate has a high probability of having a potentiality, more particularly a high potentiality, of causing invasive infection in a human);
- an *in vitro* method for determining that a *Listeria monocytogenes* isolate has a potentiality, more
20 particularly a high potentiality, of causing invasive infection in a human;
- an *in vitro* method for determining that a *Listeria monocytogenes* isolate, which belongs to one of serogroups IVb, IIb, IIa and IIc, more particularly to serogroup IVb or to serogroup IIb or to one of serogroups IIa and IIc, has a high probability of having a potentiality, more particularly a high potentiality, of causing invasive infection in a human;
- 25 - an *in vitro* method for determining whether a *Listeria monocytogenes* isolate has a high or low probability of having a potentiality, more particularly a high potentiality, of causing invasive infection in a human;
- an *in vitro* method for detecting or determining that a product intended for administration to a human (*e.g.*, *via* the oral or intra-gastric route or the intra-peritoneal route, more particularly *via*
30 the oral or intra-gastric route, such as a food or beverage intended for human consumption) is pathogenic (*i.e.*, toxic) to human (due to the presence of *Listeria monocytogenes*), more particularly pathogenic to an immunocompromised human, a pregnant woman, a fetus, a neonate, an infant, a child, or an elderly; and
- an *in vitro* method for detecting or determining that a line of production of a product intended for
35 administration to a human is contaminated (*e.g.*, at the surface of an element of the production line, or in the environment of said production line) by an isolate of *Listeria monocytogenes* that is pathogenic to human.

Said *L. monocytogenes* isolate may more particularly belong to lineage I or II of *L. monocytogenes*, more particularly to one of serogroups IVb, IIb, IIa and IIc of *L. monocytogenes*.

- 5 Throughout the application, the expression “a high probability of having a potentiality, more particularly a high potentiality, of causing invasive infection in a human” encompasses the meaning of “having a potentiality, more particularly a high potentiality, of causing invasive infection in a human”.

- 10 Similarly, the expression “a low probability of having a potentiality, more particularly a high potentiality, of causing invasive infection in a human” encompasses the meanings of “having a low potentiality of causing invasive infection in a human” and “not having a potentiality, more particularly a high potentiality, of causing invasive infection in a human”.

- 15 The oligonucleotides (primers and/or probes, more particularly primers), the sets of oligonucleotides (comprising primers and/or probes, more particularly primers), the kits, the computer program products and the computer devices of the application are especially adapted to the implementation of the methods of the application.

- Each feature or combination of features, which is described in the context of a method, equally applies to the products as such, and conversely, each feature or combination of features, which is
20 described in the context of a product, equally applies to the methods.

- The methods of the application involve detecting that one of genes i.-ix. is present in said *Listeria monocytogenes* isolate, more particularly that one of genes i.-iv., or one of genes vi. and vii., or one of genes v., viii. and ix. is present in said *L. monocytogenes* isolate.

25

Said genes i.-ix. are as follows, respectively:

- i. a gene, which is specific of *L. monocytogenes* clone CC1, wherein said CC1-specific gene is *LMOF2365_2702* (SEQ ID NO: 1) or one of the genes of SEQ ID NOs: 61-71,
- ii. a gene, which is specific of *L. monocytogenes* clone CC2, wherein said CC2-specific
30 gene is *LMOATCC19117_2417* (SEQ ID NO: 2) or one of the genes of SEQ ID NOs: 72-79,
- iii. a gene, which is specific of *L. monocytogenes* clone CC4, wherein said CC4-specific gene is *LMO312_1102* (SEQ ID NO: 3) or one of the genes of SEQ ID NOs: 80-85,
- iv. a gene, which is specific of *L. monocytogenes* clone CC6, wherein said CC6-specific gene is *LM1816_RS00050* (SEQ ID NO: 4) or one of the genes of SEQ ID NOs: 86-106,
- 35 v. a gene, which is specific of *L. monocytogenes* clone CC7, wherein said CC7-specific gene is *LMRG_02577* (SEQ ID NO: 9) or one of the genes of SEQ ID NOs: 142-149,

vi. a gene, which is specific of *L. monocytogenes* clone CC3, wherein said CC3-specific gene is *LMOSLCC2482_0307* (SEQ ID NO: 5) or one of the genes of SEQ ID NOs: 107-111,

vii. a gene, which is specific of *L. monocytogenes* clone CC5, wherein said CC5-specific gene is *M637_08170* (SEQ ID NO: 6) or one of the genes of SEQ ID NOs: 112-122,

5 viii. a gene, which is specific of *L. monocytogenes* clone(s) CC8 and/or CC16, more particularly which is specific of *L. monocytogenes* clones CC8 and CC16, wherein said CC8- and/or (and) CC16-specific gene of vii. is *LM5578_1185* (SEQ ID NO: 10) or one of the genes of SEQ ID NOs: 150-155, and

ix. a gene, which is specific of *L. monocytogenes* clone CC155, wherein said CC155-specific gene is *LMLG_RS02400* (SEQ ID NO: 8) or one of the genes of SEQ ID NOs: 129-141.

The sequences of these genes are described in the examples below, as well as in the appended sequence listing, the content of which is herein incorporated by reference.

More particularly, said genes i.-ix. are as follows, respectively:

15 i. a gene, which is specific of *L. monocytogenes* clone CC1, wherein said CC1-specific gene is *LMOF2365_2702* (SEQ ID NO: 1),

ii. a gene, which is specific of *L. monocytogenes* clone CC2, wherein said CC2-specific gene is *LMOATCC19117_2417* (SEQ ID NO: 2),

20 iii. a gene, which is specific of *L. monocytogenes* clone CC4, wherein said CC4-specific gene is *LMO312_1102* (SEQ ID NO: 3),

iv. a gene, which is specific of *L. monocytogenes* clone CC6, wherein said CC6-specific gene is *LM1816_RS00050* (SEQ ID NO: 4),

v. a gene, which is specific of *L. monocytogenes* clone CC7, wherein said CC7-specific gene is *LMRG_02577* (SEQ ID NO: 9),

25 vi. a gene, which is specific of *L. monocytogenes* clone CC3, wherein said CC3-specific gene is *LMOSLCC2482_0307* (SEQ ID NO: 5),

vii. a gene, which is specific of *L. monocytogenes* clone CC5, wherein said CC5-specific gene is *M637_08170* (SEQ ID NO: 6),

30 viii. a gene, which is specific of *L. monocytogenes* clone(s) CC8 and/or CC16, more particularly which is specific of *L. monocytogenes* clones CC8 and CC16, wherein said CC8- and/or CC16-specific gene of vii. is *LM5578_1185* (SEQ ID NO: 10), and

ix. a gene, which is specific of *L. monocytogenes* clone CC155, wherein said CC155-specific gene is *LMLG_RS02400* (SEQ ID NO: 8).

35 The presence of said gene i. is indicative that said *L. monocytogenes* isolate has a high probability of belonging to clone CC1 of *L. monocytogenes*. The absence of said gene i. is indicative that said *L. monocytogenes* isolate has a low probability of belonging to clone CC1 of *L. monocytogenes*.

The presence of said gene ii. is indicative that said *L. monocytogenes* isolate has a high probability of belonging to clone CC2 of *L. monocytogenes*. The absence of said gene ii. is indicative that said *L. monocytogenes* isolate has a low probability of belonging to clone CC2 of *L. monocytogenes*.

5 The presence of said gene iii. is indicative that said *L. monocytogenes* isolate has a high probability of belonging to clone CC4 of *L. monocytogenes*. The absence of said gene iii. is indicative that said *L. monocytogenes* isolate has a low probability of belonging to clone CC4 of *L. monocytogenes*.

10 The presence of said gene iv. is indicative that said *L. monocytogenes* isolate has a high probability of belonging to clone CC6 of *L. monocytogenes*. The absence of said gene iv. is indicative that said *L. monocytogenes* isolate has a low probability of belonging to clone CC6 of *L. monocytogenes*.

The presence of said gene v. is indicative that said *L. monocytogenes* isolate has a high probability of belonging to clone CC7 of *L. monocytogenes*. The absence of said gene v. is indicative that said *L. monocytogenes* isolate has a low probability of belonging to clone CC7 of *L. monocytogenes*.

15 The presence of said gene vi. is indicative that said *L. monocytogenes* isolate has a high probability of belonging to clone CC3 of *L. monocytogenes*. The absence of said gene vi. is indicative that said *L. monocytogenes* isolate has a low probability of belonging to clone CC3 of *L. monocytogenes*.

20 The presence of said gene vii. is indicative that said *L. monocytogenes* isolate has a high probability of belonging to clone CC5 of *L. monocytogenes*. The absence of said gene vii. is indicative that said *L. monocytogenes* isolate has a low probability of belonging to clone CC5 of *L. monocytogenes*.

25 The presence of said gene viii. is indicative that said *L. monocytogenes* isolate has a high probability of belonging to clone CC8 or CC16 of *L. monocytogenes*. The absence of said gene viii. is indicative that said *L. monocytogenes* isolate has a low probability of belonging to clone CC8 or CC16 of *L. monocytogenes*.

30 The presence of said gene ix. is indicative that said *L. monocytogenes* isolate has a high probability of belonging to clone CC155 of *L. monocytogenes*. The absence of said gene ix. is indicative that said *L. monocytogenes* isolate has a low probability of belonging to clone CC155 of *L. monocytogenes*.

35 The application relates to an *in vitro* method for *Listeria monocytogenes* clonogrouping. More particularly, the application relates to an *in vitro* method for determining that an isolate of *Listeria monocytogenes* has a high probability of belonging to clone CC1, to clone CC2, to clone CC4, to clone CC6, to clone CC7, to clone CC3, to clone CC5, to clone CC8 or CC16, or to clone CC155 of *L. monocytogenes*, wherein a high probability of belonging to one of these clones is indicative that said *L. monocytogenes* isolate has a high probability of having a potentiality, more particularly

a high potentiality, of causing invasive infection in a human, wherein said method comprises detecting that one of the said genes i.-ix. is present in said *L. monocytogenes* isolate, wherein the presence of one of said genes i.-ix. is indicative that said *L. monocytogenes* isolate has a high probability of belonging to the clone(s) that is(are) recited in i.-ix., respectively.

5 Prior to detecting one of said genes i.-ix., said method may comprise determining whether said *L. monocytogenes* isolate does or not belong to one of serogroups IVb, IIb, IIa and IIc, more particularly to serogroup IVb or to serogroup IIb or to one of serogroups IIa and IIc. Said method may then comprise:

10 when said *L. monocytogenes* isolate belongs to serogroup IVb, detecting that one of said genes i.-iv. is present in said *L. monocytogenes* isolate;

when said *L. monocytogenes* isolate belongs to serogroup IIb, said method comprises detecting that one of said genes vi. and vii. is present in said *L. monocytogenes* isolate; and

when said *L. monocytogenes* isolate belongs to serogroup IIa or IIc, said method comprises detecting that one of said genes v., viii. and ix. is present in said *L. monocytogenes* isolate.

15

Detecting that one of said genes is present can be performed by determining the presence or absence of at least one of said genes i.-ix., more particularly the presence or absence of at least one of said genes i.-iv. and/or of at least one of said genes vi.-vii. and/or at least one of said genes v., viii. and ix.,

20 more particularly the presence or absence of a plurality (or several) of said genes i.-ix., more particularly the presence or absence of said genes i.-iv. and/or of said genes vi.-vii. and/or said genes v., viii. and ix.,

more particularly the presence or absence of each of said genes i.-ix.

25 Determining the presence or absence of one or several genes can be achieved by any means that the person of average skill in the art may find appropriate.

For example, said determination can be performed by sequencing part or all of the genome of the *L. monocytogenes* isolate and by *in silico* searching for the presence or absence of said gene(s) (e.g., by using computer means to analyze the nucleic acid sequence).

30 Alternatively or complementarily, said determination can be performed by nucleic acid amplification (using primers or using primers and probes) and/or by nucleic acid hybridization (using nucleic acid probes).

The application thus relates to an *in vitro* method for determining that a *Listeria monocytogenes* isolate has a high probability of having a potentiality, more particularly a high potentiality, of causing invasive infection in a human, wherein said method comprises determining by the (clonogrouping) method of the application that said *L. monocytogenes* isolate has a high

35

probability of belonging to clone CC1, or to clone CC2, or to clone CC4, or to clone CC6, or to clone CC7, or to clone CC3, or to clone CC5, or to clone CC8 or CC16, or to clone CC155 of *L. monocytogenes*, wherein the determination that said *L. monocytogenes* isolate has a high probability of belonging to clone CC1, or to clone CC2, or to clone CC4, or to clone CC6, or to clone CC7, or to clone CC3, or to clone CC5, or to clone CC8 or CC16, or to clone CC155 of *L. monocytogenes*, is indicative that said *L. monocytogenes* isolate has a high probability of having a potentiality, more particularly a high potentiality, of causing invasive infection in a human.

Detecting that one of said genes is present is performed by determining the presence or absence of a plurality of said genes, more particularly by determining the presence or absence of a plurality of genes comprising said genes i.-iv. and/or said genes vi.-vii. and/or said genes v., viii. and ix.

The application also relates to an *in vitro* method for detecting or determining that a product intended for administration to a human, *e.g.*, *via* the oral or intra-gastric route or the intra-peritoneal route, more particularly *via* the oral or intra-gastric route, such as a food or beverage intended for human consumption is pathogenic to human (due to the presence of *Listeria monocytogenes*), which comprises determining that said product contains at least one isolate of *Listeria monocytogenes*, which has a high probability of having a potentiality, more particularly a high potentiality, of causing invasive infection in a human in accordance with the *in vitro* (clonogrouping) method of the application.

Said potentiality of causing an invasive infection is particularly high, where the human to which the product is intended to be administered, is an immunocompromised human, a pregnant woman, a fetus, a neonate, an infant, a child, or an elderly.

The application also relates to an *in vitro* method for detecting or determining that a line of production of a product intended for administration to a human is contaminated (*e.g.*, at the surface of an element of the production line or at a surface in the environment surrounding the production line) by an isolate of *Listeria monocytogenes* that is pathogenic to human, which comprises collecting a (biological) sample from said production line (*e.g.*, from the surface of an element of said production), wherein said sample is a sample of

- the raw material(s) that is(are) implemented as starting product(s) of the production process of said product intended for administration to a human and/or of
- the intermediate product(s) that are produced in the course of said production process, and/or of
- the final product(s) that are produced by said production process,

and/or wherein said sample is

- the sample of a (starting, intermediate or final) product contact surface (*e.g.*, the brine cooling system, casing or wiener peeling equipment (peelers, table tops, hoppers), product conveyors, the

device power supply of the slicer, the blade of the slicer, the packing machines, the supports, the carriages, the containers, the balances, and knives) and/or

- the sample of a surface surrounding the production line (*e.g.*, the ground or floor onto which said production line is positioned or built),

5 and determining that said sample contains at least one isolate of *Listeria monocytogenes*, which has a high probability of having a potentiality, more particularly a high potentiality, of causing invasive infection in a human in accordance with the *in vitro* (clonogrouping) method of the application,

wherein the determination that said sample contains at least one isolate of *Listeria monocytogenes*,
10 which has a high probability of having a potentiality, more particularly a high potentiality, of causing invasive infection in a human is indicative that said production line is contaminated with an isolate of *Listeria monocytogenes* that is pathogenic to human.

Said potentiality of causing an invasive infection is particularly high, where the human to which the product is intended to be administered, is an immunocompromised human, a pregnant woman,
15 a fetus, a neonate, an infant, a child, or an elderly.

More particularly, the application relates to an *in vitro* method for determining that a *Listeria monocytogenes* isolate, which belongs to one of serogroups IVb, IIb, IIa and IIc, more particularly to serogroup IVb or to serogroup IIb or to one of serogroups IIa and IIc, has a high probability of
20 having a potentiality, more particularly a high potentiality, of causing invasive infection in a human, wherein said method comprises

when said *L. monocytogenes* isolate belongs to serogroup IVb, detecting that one of genes i.-iv. is present in said *L. monocytogenes* isolate,

when said *L. monocytogenes* isolate belongs to serogroup IIb, detecting that one of genes vi. and
25 vii. is present in said *L. monocytogenes* isolate,

when said *L. monocytogenes* isolate belongs to one of serogroups IIa and IIc, detecting that one of genes v., viii. and ix. is present in said *L. monocytogenes* isolate;

said genes i.-iv., said genes vi. and vii., and said genes v., viii. and ix. being as above-defined,

wherein detecting that one of said genes i.-iv., or of said genes vi. and vii., or of said genes v., viii.
30 and ix., respectively, is present in said *L. monocytogenes* isolate is indicative that said *L. monocytogenes* isolate has a high probability of having a potentiality, more particularly a high potentiality, of causing invasive infection in a human.

In addition to detecting that one of genes v., viii. and ix. is present in said *L. monocytogenes*
35 isolate when said *L. monocytogenes* isolate belongs to one of serogroups IIa and IIc, said method may further comprise determining whether one or none of the further following genes is or are present:

x. a gene, which is specific of *L. monocytogenes* clone CC121, wherein said CC121-specific gene is *LM6179_0610* (SEQ ID NO: 7) or one of the genes of SEQ ID NOs: 123-128, more particularly *LM6179_0610* (SEQ ID NO: 7); and

xi. a gene, which is specific of *L. monocytogenes* clone CC9, wherein said CC9-specific
5 gene is *lmo1118* (SEQ ID NO: 11),

wherein detecting that one of said genes i.-iv., or of said genes vi. and vii., or of said genes v., viii. and ix., respectively, is present in said *L. monocytogenes* isolate is indicative that said *L. monocytogenes* isolate has a high probability of having a potentiality, more particularly a high potentiality, of causing invasive infection in a human, and

10 wherein detecting that one of said genes x. and xi., is present in said *L. monocytogenes* isolate is indicative that said *L. monocytogenes* isolate has a low probability of having a potentiality, more particularly a high potentiality, of causing invasive infection in a human.

The application also relates to an *in vitro* method for determining whether a *Listeria*
15 *monocytogenes* isolate has a high or low probability of having a potentiality, more particularly a high potentiality, of causing invasive infection in a human, wherein said method comprises determining whether one or none of genes i.-ix. is or are present in said *L. monocytogenes* isolate, said genes i.-ix. being as above-defined; and

when none of genes i.-ix. are present in said *L. monocytogenes* isolate, determining whether said
20 *L. monocytogenes* isolate does or not belong to (lineage I or II of *L. monocytogenes*, more particularly to) one of serogroups IVb, IIb, IIa and IIc, more particularly to serogroup IVb or to serogroup IIb or to one of serogroups IIa and IIc;

wherein determining that one of said genes i.-ix. is present in said *L. monocytogenes* isolate is indicative that said *L. monocytogenes* isolate has a high probability of having a potentiality, more
25 particularly a high potentiality, of causing invasive infection in a human,

wherein determining that none of said genes i.-ix. are present in said *L. monocytogenes* isolate and determining that said *L. monocytogenes* isolate belongs to (lineage I of *L. monocytogenes*, more particularly to) one of serogroups IVb and IIb, is indicative that said *L. monocytogenes* isolate has a high probability of having a potentiality, more particularly a high potentiality, of causing
30 invasive infection in a human,

wherein determining that none of said genes i.-ix. are present in said *L. monocytogenes* isolate and determining that said *L. monocytogenes* isolate belongs to (lineage II of *L. monocytogenes*, more particularly to) one of serogroups IIa and IIc, is indicative that said *L. monocytogenes* isolate has a low probability of having a potentiality, more particularly a high potentiality, of causing invasive
35 infection in a human, and

wherein determining that none of said genes i.-ix. are present in that said *L. monocytogenes* isolate and determining that said *L. monocytogenes* isolate does not belong to (lineage I or II of *L.*

monocytogenes, more particularly to) any of serogroups IVb, IIb, IIa and IIc is indicative that said *L. monocytogenes* isolate has a low probability of having a potentiality, more particularly a high potentiality, of causing invasive infection in a human.

- 5 In addition to determining whether one or none of said genes i.-ix. is or are present, said method may further comprise determining whether one or none of the further following genes is or are present:

x. a gene, which is specific of *L. monocytogenes* clone CC121, wherein said CC121-specific gene is *LM6179_0610* (SEQ ID NO: 7) or one of the genes of SEQ ID NOs: 123-128,
10 more particularly *LM6179_0610* (SEQ ID NO: 7); and

xi. a gene, which is specific of *L. monocytogenes* clone CC9, wherein said CC9-specific gene is *lmo1118* (SEQ ID NO: 11),

wherein determining that one of said genes i.-ix. is present in said *L. monocytogenes* isolate is indicative that said *L. monocytogenes* isolate has a high probability of having a potentiality, more
15 particularly a high potentiality, of causing invasive infection in a human,

wherein determining that none of said genes i.-xi. are present in said *L. monocytogenes* isolate and determining that said *L. monocytogenes* isolate belongs to (lineage I of *L. monocytogenes*, more particularly to) one of serogroups IVb and IIb, is indicative that said *L. monocytogenes* isolate has a high probability of having a potentiality, more particularly a high potentiality, of causing invasive
20 infection in a human,

wherein determining that one of said genes x.-xi. is present in said *L. monocytogenes* isolate is indicative that said *L. monocytogenes* isolate has a low probability of having a potentiality, more particularly a high potentiality, of causing invasive infection in a human,

wherein determining that none of said genes i.-xi. are present in said *L. monocytogenes* isolate and
25 determining that said *L. monocytogenes* isolate belongs to (lineage II of *L. monocytogenes*, more particularly to) one of serogroups IIa and IIc, is indicative that said *L. monocytogenes* isolate has a low probability of having a potentiality, more particularly a high potentiality, of causing invasive infection in a human, and

wherein determining that none of said genes i.-xi. are present in that said *L. monocytogenes* isolate
30 and determining that said *L. monocytogenes* isolate does not belong to (lineage I or II of *L. monocytogenes*, more particularly to) any of serogroups IVb, IIb, IIa and IIc is indicative that said *L. monocytogenes* isolate has a low probability of having a potentiality, more particularly a high potentiality, of causing invasive infection in a human.

- 35 Determining whether said *L. monocytogenes* isolate does or not belong to one of serogroups IVb, IIb, IIa and IIc, more particularly to serogroup IVb or to serogroup IIb or to one of serogroups IIa

and IIc, can be performed as described in Doumith *et al.*, 2004, the content of which is herein incorporated by reference.

Hence, determining whether said *L. monocytogenes* isolate does or not belong to one of serogroups IVb, IIb, IIa and IIc, more particularly to serogroup IVb, or to serogroup IIb, or to one of
5 serogroups IIa and IIc, can be performed by determining the presence or absence of serogroup marker genes, said serogroup marker genes comprising *L. monocytogenes* genes *lmo0737*, *lmo1118* (SEQ ID NO: 11), ORF2819 and ORF2110.

Determining the presence or absence of said serogroup marker genes is performed by nucleic acid amplification using “serogroup” primers for (specific) nucleic acid amplification from each of said
10 serogroup marker genes. Said “serogroup” primers can be primers, which produce from marker gene *lmo0737* an amplification product of 691bp, from marker gene *lmo1118* (SEQ ID NO: 11) an amplification product of 906 bp, from marker gene ORF2819 an amplification product of 471 bp, and from marker gene ORF2110 an amplification product of 597 bp.

15 Said “serogroup” primers may comprise

- the primer pair of SEQ ID NO: 182 and 183 (for nucleic acid amplification from marker gene *lmo0737*), or a primer pair, which anneals to the marker gene *lmo0737* at the same positions as said primer pair of SEQ ID NO: 182 and 183 and which amplifies from this marker gene the same target region as said primer pair of SEQ ID NO: 182 and 183,
- 20 - the primer pair of SEQ ID NOs: 47 and 48 (for nucleic acid amplification from marker gene *lmo1118* (SEQ ID NO: 11)), or a primer pair, which anneals to the marker gene *lmo1118* (SEQ ID NO: 11) at the same positions as said primer pair of SEQ ID NOs: 47 and 48 and which amplifies from this marker gene the same target region as said primer pair of SEQ ID NOs: 47 and 48,
- the primer pair of SEQ ID NOs: 184 and 185 (for nucleic acid amplification from marker gene
25 ORF2819), or a primer pair, which anneals to the marker gene at the same positions as said primer pair of SEQ ID NOs: 184 and 185 and which amplifies from this marker gene the same target region as said primer pair of SEQ ID NOs: 184 and 185, and
- the primer pair of SEQ ID NOs: 186 and 187 (for nucleic acid amplification from marker gene ORF2110), or a primer pair, which anneals to the marker gene ORF2110 at the same positions as
30 said primer pair of SEQ ID NOs: 186 and 187 and which amplifies from this marker gene the same target region as said primer pair of SEQ ID NOs: 186 and 187.

A primer pair, which anneals to the marker gene *lmo0737* at the same positions as said primer pair of SEQ ID NO: 182 and 183 and which amplifies from this marker gene the same target region as said primer pair of SEQ ID NO: 182 and 183, may consist of a primer pair, wherein a first
35 oligonucleotide is of the same length as the primer of SEQ ID NO: 182 and is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 182, and wherein the second oligonucleotide is of the same length as the primer of SEQ

ID NO: 183 and is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 183.

A primer pair, which anneals to the marker gene *lmo1118* (SEQ ID NO: 11) at the same positions as said primer pair of SEQ ID NOs: 47 and 48 and which amplifies from this marker gene the same target region as said primer pair of SEQ ID NOs: 47 and 48, may consist of a primer pair, wherein a first oligonucleotide is of the same length as the primer of SEQ ID NO: 47 and is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 47, and wherein the second oligonucleotide is of the same length as the primer of SEQ ID NO: 48 and is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 48.

A primer pair, which anneals to the marker gene ORF2819 at the same positions as said primer pair of SEQ ID NOs: 184 and 185 and which amplifies from this marker gene the same target region as said primer pair of SEQ ID NOs: 184 and 185, may consist of a primer pair, wherein a first oligonucleotide is of the same length as the primer of SEQ ID NO: 184 and is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 184, and wherein the second oligonucleotide is of the same length as the primer of SEQ ID NO: 185 and is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 185.

A primer pair, which anneals to the marker gene ORF2110 at the same positions as said primer pair of SEQ ID NOs: 186 and 187 and which amplifies from this marker gene the same target region as said primer pair of SEQ ID NOs: 186 and 187, may consist of a primer pair, wherein a first oligonucleotide is of the same length as the primer of SEQ ID NO: 186 and is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 186, and wherein the second oligonucleotide is of the same length as the primer of SEQ ID NO: 187 and is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 187.

Said “serogroup” marker genes may further be used with a *Listeria* genus marker gene, such as the *L. monocytogenes prs* gene. Determining the presence or absence of said *L. monocytogenes prs* marker gene can be performed by nucleic acid amplification using primers for nucleic acid amplification from gene *prs*. The *prs* primers may amplify a target region of 370 bp from marker gene *prs*. The *prs* primers may comprise the primer pair of SEQ ID NO: 188 and 189 for nucleic acid amplification from marker gene *prs*, or a primer pair, which anneals to the marker gene *prs* at the same positions as said primer pair of SEQ ID NO: 188 and 189 and which amplifies from this marker gene the same target region as said primer pair of SEQ ID NO: 188 and 189.

35

The application thus involve

detecting that one of said genes i.-ix., or said genes i.-x., or said genes i.-xi., or said genes i.-ix. and xi., more particularly of genes i.-iv. and/or genes vi.-vii. and/or genes v., viii. and ix., is present in said *L. monocytogenes* isolate, or

5 determining whether one or none of said genes i.-ix., or said genes i.-x., or said genes i.-xi., or said genes i.-ix. and xi., more particularly of genes i.-iv. and/or genes vi.-vii. and/or genes v., viii. and ix., is or are present in said *L. monocytogenes* isolate.

10 Detecting that one of said genes is present or determining whether one or none of said genes is or are present can be performed by determining the presence or absence of a plurality of said genes, more particularly by determining the presence or absence of a plurality of genes comprising said genes i.-iv. and/or said genes vi.-vii. and/or said genes v., viii. and ix.

15 In the application, a plurality (or several) of genes encompasses at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, the 9 of said genes i.-ix., more particularly at least 2 of said genes i.-ix., at least 3, at least 4, more particularly 2, 3 or 4 of said genes i.-ix. For example, a plurality (or several) of genes encompasses at least 2, 3 or the 4 of said genes i.-iv. For example, a plurality (or several) of genes encompasses at least 2 or the 3 of said genes v., viii. and ix. For example, a plurality (or several) of genes encompasses the two of said genes vi. and vii.

Hence, said plurality of genes
 20 comprises said genes i.-iv., or
 comprises said genes vi.-vii., or
 comprises said genes v., viii. and ix., or
 comprises said genes v., and viii.-xi., or
 comprises said genes i.-iv. and said genes vi.-vii., or
 25 comprises said genes i.-iv. and said genes v., viii. and ix., or
 comprises said genes i.-iv. and said genes v., and viii.-xi., or
 comprises said genes vi.-vii. and said genes v., viii. and ix., or
 comprises said genes vi.-vii. and said genes v., and viii.-xi., or
 comprises said genes i.-iv., said genes vi.-vii. and said genes v., viii. and ix., or
 30 comprises said genes i.-iv., said genes vi.-vii. and said genes v., and viii.-xi.

The presence or absence of each gene of said plurality can be determined simultaneously and/or sequentially.

35 Detecting that one of said genes is present (or determining whether one or none of said genes is or are present) can be performed by simultaneously and/or sequentially (more particularly simultaneously) determining the presence or absence of at least one of said genes i.-ix., more particularly of at least one of said genes i.-iv. and/or of at least one of said genes vi.-vii. and/or at

least one of said genes v., viii. and ix., more particularly of several of said genes i.-ix., more particularly of each of said genes i.-ix.

Said determination can be performed simultaneously and/or sequentially (more particularly simultaneously). Each and every combination of simultaneous determination and/or sequential
5 determination is explicitly encompassed in the application.

For example, the presence or absence of several (*e.g.*, 2, 3 or 4) of said genes i.-ix. may be determined simultaneously, and the presence or absence of the other of said genes i.-ix. may be determined sequentially, *i.e.*, before or after (more particularly after) said simultaneous detection. The presence or absence of said other genes i.-ix. may be in turn determined by one or several
10 other simultaneous detection(s) (*e.g.*, two separate simultaneous detections).

For example:

- the presence or absence of said genes i.-iv. can be determined simultaneously,
- the presence or absence of said genes vi.-vii. can be determined simultaneously,
- the presence or absence of said genes v., viii. and ix. can be determined simultaneously, and
15 - each of these three simultaneous determinations can be performed simultaneously or sequentially with respect to each other.

Said determination can be performed in separate or common containers, *e.g.*, in separate or common

- tube(s) or multiwell plate(s) suitable for nucleic acid amplification (*i.e.*, suitable for
20 receiving a reaction mixture containing oligonucleotides, *e.g.*, primers, or primers and probes, for nucleic acid amplification), or

- solid support(s), such as array(s) (*e.g.*, macro-array(s), micro-array(s) or nano-array(s)), chip(s) or microbeads, wherein said solid support(s) is(are) suitable for nucleic acid hybridization or for sequencing (and wherein said solid support(s) may contain oligonucleotides, *e.g.*, probes,
25 (covalently) linked thereto).

More particularly, the presence or absence of each gene of said plurality is determined in or on the same container. For example, the simultaneous determination of the presence or absence of said genes i.-iv. can be performed in or on a first container (*e.g.*, a first tube suitable for nucleic acid amplification), which is distinct from a second container (*e.g.*, a second tube suitable for nucleic
30 acid amplification) in or on which the simultaneous determination of the presence or absence of said genes vi.-vii. is performed, and which is also distinct from a third container (*e.g.*, a third tube suitable for nucleic acid amplification) in or on which the simultaneous determination of the presence or absence of said genes v., viii. and ix. is performed.

Said container(s) may contain reagents (*e.g.*, primers, or probes or primers and probes) suitable for
35 determining the presence or absence of each of said genes.

Primers and/or probes can be used to determine the presence or absence of each gene of said plurality. These primers and/or probes advantageously are

- primer pairs,
- primer pairs and probes, or

5 - probes,

more particularly primer pairs,

wherein each primer pair, or primer pair and probe, or probe, is specific of a (different) gene of said plurality, thereby providing a set of primer pairs, or primer pairs and probes, or probes, which (specifically) detects the whole range of genes of said plurality.

10 When the presence or absence of each gene of said plurality is determined in or on the same container, said container may thus contain these primers and/or probes.

Said primers may comprise at least one primer pair suitable for nucleic acid amplification from one of genes i.-ix., more particularly at least one first primer pair suitable for nucleic acid
15 amplification from one of genes i.-ix. and at least one second primer pair suitable for nucleic acid amplification from another of genes i.-ix.

Said primers may comprise several primer pairs, wherein each primer pair is suitable for nucleic acid amplification from different genes, said different genes being selected from genes i.-ix.

More particularly, said primers may comprise several primer pairs, wherein each primer pair is
20 suitable for nucleic acid amplification from different genes, said different genes being selected from genes i.-iv. and/or from genes vi.-vii. and/or from genes v., viii. and ix.

More particularly, said primers may comprise several primer pairs, wherein each primer pair is suitable for nucleic acid amplification from different genes, said different genes being each one of genes i.-iv. and/or each one of genes vi.-vii. and/or each one of genes v., viii. and ix.

25 More particularly, said primers may comprise several primer pairs, wherein each primer pair is suitable for nucleic acid amplification from different genes, said different genes being each of genes i.-ix.

Said nucleic acid amplification is specific of each of said genes, more particularly of each of said genes i.-iv. and/or each of said genes vi.-vii. and/or each of said genes v., viii. and ix. Hence, the
30 sequences of a primer pair are such that the presence of an amplification product (*i.e.*, of an amplicon produced using the primer pair in an amplification reaction) is indicative that said gene is present and, conversely, the absence of amplification product is indicative that said gene is absent. In other words, the primer pair is specific of the gene.

Advantageously, said genes i.-iv. are the genes of SEQ ID NOs: 1-4 and/or of said genes vi.-vii.
35 are the genes of SEQ ID NOs: 5-6 and/or said genes v., viii. and ix. are the genes of SEQ ID NOs: 9, 10 and 8, respectively.

The typical length of a primer is 15-30 nucleotides. In the application, a primer consists of 15-30 nucleotides, more particularly 19-25 nucleotides, more particularly of 20-24 nucleotides, *i.e.*, of 20, 21, 22, 23 or 24 nucleotides.

The Guanine + Cytosine (G+C) content of a primer typically is of 40-60%.

5

For example, for specific amplification from said gene i. of SEQ ID NO: 1 (clone CC1), said primers may comprise

- the primer pair of SEQ ID NO: 17 and 18, or
- a primer pair, which still is specific of gene i. of SEQ ID NO: 1 and which anneals to gene i. of SEQ ID NO: 1 at positions that differ by ± 5 nucleotides (more particularly by ± 4 nucleotides, by ± 3 nucleotides, by ± 2 nucleotides, by ± 1 nucleotides or by 0 nucleotide) from the positions at which the primer pair of SEQ ID NOs: 17 and 18 anneals to gene i. of SEQ ID NO: 1; or
- a primer pair, which still is specific of gene i. of SEQ ID NO: 1, and which anneals to gene i. of SEQ ID NO: 1 to amplify

- 15 a fragment from gene i. of SEQ ID NO: 1, wherein said fragment comprises the (target) sequence SEQ ID NO: 16 that is amplified from gene i. of SEQ ID NO: 1 by the primer pair of SEQ ID NOs: 17 and 18 (*i.e.*, the target sequence of SEQ ID NO: 16) and is at most 10 nucleotides longer than said target sequence (more particularly at most 9, 8, 7, 6, 5, 4, 3, 2, 1 or 0 nucleotide(s) longer than said target sequence); or

- 20 a fragment from gene i. of SEQ ID NO: 1, wherein said fragment consists of the (target) sequence that is amplified from gene i. of SEQ ID NO: 1 by the primer pair of SEQ ID NOs: 17 and 18 (*i.e.*, the sequence of SEQ ID NO: 16) but deleted from at most 10 nucleotides (more particularly at most 9, 8, 7, 6, 5, 4, 3, 2, 1 or 0 nucleotide(s)); or

- a primer pair, which anneals to gene i. of SEQ ID NO: 1 to specifically amplify (from said gene) the same target sequence as said primer pair of SEQ ID NOs: 17 and 18 (*i.e.*, the sequence of SEQ ID NO: 16), wherein the first primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 17 and the second primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 18.

- 30 More particularly, for specific amplification from said gene i. of SEQ ID NO: 1, said primers may comprise a primer pair for specifically amplifying (from said gene) the same target sequence as the primer pair of SEQ ID NOs: 17 and 18 (*i.e.*, the target sequence of SEQ ID NO: 16).

- More particularly, for specific amplification from said gene i. of SEQ ID NO: 1, said primers may comprise a primer pair for specifically amplifying (from said gene) the same target sequence as the primer pair of SEQ ID NOs: 17 and 18 (*i.e.*, the target sequence of SEQ ID NO: 16), wherein said primer pair is

- the primer pair of SEQ ID NOs: 17 and 18, or

- a primer pair, which anneals to gene i. of SEQ ID NO: 1 to specifically amplify (from said gene) the same target sequence as said primer pair of SEQ ID NOs: 17 and 18 (*i.e.*, the target sequence of SEQ ID NO: 16), wherein the first primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 17 and the second primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 18.

Each of the first and second primers of the primer pair may independently consist of 15-30 nucleotides, more particularly 19-25 nucleotides, more particularly of 20-24 nucleotides, *i.e.*, of 20, 21, 22, 23 or 24 nucleotides. More particularly, the first nucleotide of the primer pair may be of the same nucleotide length as the primer of SEQ ID NO: 17 and the second nucleotide may be of the same nucleotide length as the primer of SEQ ID NO: 18. The GC content of each of said first and second primers may independently be of 40-60%.

For example, for specific amplification from said gene ii. of SEQ ID NO: 2 (clone CC2), said primers may comprise

- the primer pair of SEQ ID NO: 20 and 21, or
- a primer pair, which still is specific of gene ii. of SEQ ID NO: 2 and which anneals to gene ii. of SEQ ID NO: 2 at positions that differ by ± 5 nucleotides (more particularly by ± 4 nucleotides, by ± 3 nucleotides, by ± 2 nucleotides, by ± 1 nucleotides or by 0 nucleotide) from the positions at which the primer pair of SEQ ID NOs: 20 and 21 anneals to gene ii. of SEQ ID NO: 2; or
- a primer pair, which still is specific of gene ii. of SEQ ID NO: 2, and which anneals to gene ii. of SEQ ID NO: 2 to amplify

a fragment from gene ii. of SEQ ID NO: 2, wherein said fragment comprises the (target) sequence SEQ ID NO: 19 that is amplified from gene ii. of SEQ ID NO: 2 by the primer pair of SEQ ID NOs: 20 and 21 (*i.e.*, the target sequence of SEQ ID NO: 19) and is at most 10 nucleotides longer than said target sequence (more particularly at most 9, 8, 7, 6, 5, 4, 3, 2, 1 or 0 nucleotide(s) longer than said target sequence); or

a fragment from gene ii. of SEQ ID NO: 2, wherein said fragment consists of the (target) sequence that is amplified from gene ii. of SEQ ID NO: 2 by the primer pair of SEQ ID NOs: 20 and 21 (*i.e.*, the sequence of SEQ ID NO: 19) but deleted from at most 10 nucleotides (more particularly at most 9, 8, 7, 6, 5, 4, 3, 2, 1 or 0 nucleotide(s)); or

- a primer pair, which anneals to gene ii. of SEQ ID NO: 2 to specifically amplify (from said gene) the same target sequence as said primer pair of SEQ ID NOs: 20 and 21 (*i.e.*, the sequence of SEQ ID NO: 19), wherein the first primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 20 and the second primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 21.

More particularly, for specific amplification from said gene ii. of SEQ ID NO: 2, said primers may comprise a primer pair for specifically amplifying (from said gene) the same target sequence as the primer pair of SEQ ID NOs: 20 and 21 (*i.e.*, the target sequence of SEQ ID NO: 19).

More particularly, for specific amplification from said gene ii. of SEQ ID NO: 2, said primers may
5 comprise a primer pair for specifically amplifying (from said gene) the same target sequence as the primer pair of SEQ ID NOs: 20 and 21 (*i.e.*, the target sequence of SEQ ID NO: 19), wherein said primer pair is

- the primer pair of SEQ ID NOs: 20 and 21, or
- a primer pair, which anneals to gene ii. of SEQ ID NO: 2 to specifically amplify (from said gene)
10 the same target sequence as said primer pair of SEQ ID NOs: 20 and 21 (*i.e.*, the target sequence of SEQ ID NO: 19), wherein the first primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 20 and the second primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 21.
- 15 Each of the first and second primers of the primer pair may independently consist of 15-30 nucleotides, more particularly 19-25 nucleotides, more particularly of 20-24 nucleotides, *i.e.*, of 20, 21, 22, 23 or 24 nucleotides. More particularly, the first nucleotide of the primer pair may be of the same nucleotide length as the primer of SEQ ID NO: 20 and the second nucleotide may be of the same nucleotide length as the primer of SEQ ID NO: 21. The GC content of each of said
20 first and second primers may independently be of 40-60%.

For example, for specific amplification from said gene iii. of SEQ ID NO: 3 (clone CC4), said primers may comprise

- the primer pair of SEQ ID NO: 23 and 24, or
- 25 - a primer pair, which still is specific of gene iii. of SEQ ID NO: 3 and which anneals to gene iii. of SEQ ID NO: 3 at positions that differ by ± 5 nucleotides (more particularly by ± 4 nucleotides, by ± 3 nucleotides, by ± 2 nucleotides, by ± 1 nucleotides or by 0 nucleotide) from the positions at which the primer pair of SEQ ID NOs: 23 and 24 anneals to gene iii. of SEQ ID NO: 3; or
- a primer pair, which still is specific of gene iii. of SEQ ID NO: 3, and which anneals to gene iii.
30 of SEQ ID NO: 3 to amplify
 - a fragment from gene iii. of SEQ ID NO: 3, wherein said fragment comprises the (target) sequence SEQ ID NO: 22 that is amplified from gene iii. of SEQ ID NO: 3 by the primer pair of SEQ ID NOs: 23 and 24 (*i.e.*, the target sequence of SEQ ID NO: 22) and is at most 10 nucleotides longer than said target sequence (more particularly at most 9, 8, 7, 6, 5, 4, 3,
35 2, 1 or 0 nucleotide(s) longer than said target sequence); or
 - a fragment from gene iii. of SEQ ID NO: 3, wherein said fragment consists of the (target) sequence that is amplified from gene iii. of SEQ ID NO: 3 by the primer pair of SEQ ID

- NOs: 23 and 24 (*i.e.*, the sequence of SEQ ID NO: 22) but deleted from at most 10 nucleotides (more particularly at most 9, 8, 7, 6, 5, 4, 3, 2, 1 or 0 nucleotide(s)); or
- a primer pair, which anneals to gene iii. of SEQ ID NO: 3 to specifically amplify (from said gene) the same target sequence as said primer pair of SEQ ID NOs: 23 and 24 (*i.e.*, the sequence of SEQ ID NO: 22), wherein the first primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 23 and the second primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 24.
- More particularly, for specific amplification from said gene iii. of SEQ ID NO: 3, said primers may comprise a primer pair for specifically amplifying (from said gene) the same target sequence as the primer pair of SEQ ID NOs: 23 and 24 (*i.e.*, the target sequence of SEQ ID NO: 22).
- More particularly, for specific amplification from said gene iii. of SEQ ID NO: 3, said primers may comprise a primer pair for specifically amplifying (from said gene) the same target sequence as the primer pair of SEQ ID NOs: 23 and 24 (*i.e.*, the target sequence of SEQ ID NO: 22), wherein said primer pair is
- the primer pair of SEQ ID NOs: 23 and 24, or
 - a primer pair, which anneals to gene iii. of SEQ ID NO: 3 to specifically amplify (from said gene) the same target sequence as said primer pair of SEQ ID NOs: 23 and 24 (*i.e.*, the target sequence of SEQ ID NO: 22), wherein the first primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 23 and the second primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 24.
- Each of the first and second primers of the primer pair may independently consist of 15-30 nucleotides, more particularly 19-25 nucleotides, more particularly of 20-24 nucleotides, *i.e.*, of 20, 21, 22, 23 or 24 nucleotides. More particularly, the first nucleotide of the primer pair may be of the same nucleotide length as the primer of SEQ ID NO: 23 and the second nucleotide may be of the same nucleotide length as the primer of SEQ ID NO: 24. The GC content of each of said first and second primers may independently be of 40-60%.
- For example, for specific amplification from said gene iv. of SEQ ID NO: 4 (clone CC6), said primers may comprise
- the primer pair of SEQ ID NO: 26 and 27, or
 - a primer pair, which still is specific of gene iv. of SEQ ID NO: 4 and which anneals to gene iv. of SEQ ID NO: 4 at positions that differ by ± 5 nucleotides (more particularly by ± 4 nucleotides, by ± 3 nucleotides, by ± 2 nucleotides, by ± 1 nucleotides or by 0 nucleotide) from the positions at which the primer pair of SEQ ID NOs: 26 and 27 anneals to gene iv. of SEQ ID NO: 4; or

- a primer pair, which still is specific of gene iv. of SEQ ID NO: 4, and which anneals to gene iv. of SEQ ID NO: 4 to amplify

5 a fragment from gene iv. of SEQ ID NO: 4, wherein said fragment comprises the (target) sequence SEQ ID NO: 25 that is amplified from gene iv. of SEQ ID NO: 4 by the primer pair of SEQ ID NOs: 26 and 27 (*i.e.*, the target sequence of SEQ ID NO: 25) and is at most 10 nucleotides longer than said target sequence (more particularly at most 9, 8, 7, 6, 5, 4, 3, 2, 1 or 0 nucleotide(s) longer than said target sequence); or

10 a fragment from gene iv. of SEQ ID NO: 4, wherein said fragment consists of the (target) sequence that is amplified from gene iv. of SEQ ID NO: 4 by the primer pair of SEQ ID NOs: 26 and 27 (*i.e.*, the sequence of SEQ ID NO: 25) but deleted from at most 10 nucleotides (more particularly at most 9, 8, 7, 6, 5, 4, 3, 2, 1 or 0 nucleotide(s)); or

- a primer pair, which anneals to gene iv. of SEQ ID NO: 4 to specifically amplify (from said gene) the same target sequence as said primer pair of SEQ ID NOs: 26 and 27 (*i.e.*, the sequence of SEQ ID NO: 25), wherein the first primer of said primer pair is at least 95% (more particularly 15 at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 26 and the second primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 27.

More particularly, for specific amplification from said gene iv. of SEQ ID NO: 4, said primers may comprise a primer pair for specifically amplifying (from said gene) the same target sequence 20 as the primer pair of SEQ ID NOs: 26 and 27 (*i.e.*, the target sequence of SEQ ID NO: 25).

More particularly, for specific amplification from said gene iv. of SEQ ID NO: 4, said primers may comprise a primer pair for specifically amplifying (from said gene) the same target sequence as the primer pair of SEQ ID NOs: 26 and 27 (*i.e.*, the target sequence of SEQ ID NO: 25), wherein said primer pair is

25 - the primer pair of SEQ ID NOs: 26 and 27, or

- a primer pair, which anneals to gene iv. of SEQ ID NO: 4 to specifically amplify (from said gene) the same target sequence as said primer pair of SEQ ID NOs: 26 and 27 (*i.e.*, the target sequence of SEQ ID NO: 25), wherein the first primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ 30 ID NO: 26 and the second primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 27.

Each of the first and second primers of the primer pair may independently consist of 15-30 nucleotides, more particularly 19-25 nucleotides, more particularly of 20-24 nucleotides, *i.e.*, of 20, 21, 22, 23 or 24 nucleotides. More particularly, the first nucleotide of the primer pair may be 35 of the same nucleotide length as the primer of SEQ ID NO: 26 and the second nucleotide may be of the same nucleotide length as the primer of SEQ ID NO: 27. The GC content of each of said first and second primers may independently be of 40-60%.

For example, for specific amplification from said gene vi. of SEQ ID NO: 5 (clone CC3), said primers may comprise

- the primer pair of SEQ ID NO: 29 and 30, or
- 5 - a primer pair, which still is specific of gene vi. of SEQ ID NO: 5 and which anneals to gene vi. of SEQ ID NO: 5 at positions that differ by ± 5 nucleotides (more particularly by ± 4 nucleotides, by ± 3 nucleotides, by ± 2 nucleotides, by ± 1 nucleotides or by 0 nucleotide) from the positions at which the primer pair of SEQ ID NOs: 29 and 30 anneals to gene vi. of SEQ ID NO: 5; or
- a primer pair, which still is specific of gene vi. of SEQ ID NO: 5, and which anneals to gene vi.
- 10 of SEQ ID NO: 5 to amplify
 - a fragment from gene vi. of SEQ ID NO: 5, wherein said fragment comprises the (target) sequence SEQ ID NO: 28 that is amplified from gene vi. of SEQ ID NO: 5 by the primer pair of SEQ ID NOs: 29 and 30 (*i.e.*, the target sequence of SEQ ID NO: 28) and is at most 10 nucleotides longer than said target sequence (more particularly at most 9, 8, 7, 6, 5, 4, 3,
 - 15 2, 1 or 0 nucleotide(s) longer than said target sequence); or
 - a fragment from gene vi. of SEQ ID NO: 5, wherein said fragment consists of the (target) sequence that is amplified from gene vi. of SEQ ID NO: 5 by the primer pair of SEQ ID NOs: 29 and 30 (*i.e.*, the sequence of SEQ ID NO: 28) but deleted from at most 10 nucleotides (more particularly at most 9, 8, 7, 6, 5, 4, 3, 2, 1 or 0 nucleotide(s)); or
- 20 - a primer pair, which anneals to gene vi. of SEQ ID NO: 5 to specifically amplify (from said gene) the same target sequence as said primer pair of SEQ ID NOs: 29 and 30 (*i.e.*, the sequence of SEQ ID NO: 28), wherein the first primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 29 and the second primer of said primer pair is at least 95% (more particularly at least 96%, at least
- 25 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 30.

More particularly, for specific amplification from said gene vi. of SEQ ID NO: 5, said primers may comprise a primer pair for specifically amplifying (from said gene) the same target sequence as the primer pair of SEQ ID NOs: 29 and 30 (*i.e.*, the target sequence of SEQ ID NO: 28).

- More particularly, for specific amplification from said gene vi. of SEQ ID NO: 5, said primers
- 30 may comprise a primer pair for specifically amplifying (from said gene) the same target sequence as the primer pair of SEQ ID NOs: 29 and 30 (*i.e.*, the target sequence of SEQ ID NO: 28), wherein said primer pair is

- the primer pair of SEQ ID NOs: 29 and 30, or
- a primer pair, which anneals to gene vi. of SEQ ID NO: 5 to specifically amplify (from said
- 35 gene) the same target sequence as said primer pair of SEQ ID NOs: 29 and 30 (*i.e.*, the target sequence of SEQ ID NO: 28), wherein the first primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ

ID NO: 29 and the second primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 30.

Each of the first and second primers of the primer pair may independently consist of 15-30 nucleotides, more particularly 19-25 nucleotides, more particularly of 20-24 nucleotides, *i.e.*, of 20, 21, 22, 23 or 24 nucleotides. More particularly, the first nucleotide of the primer pair may be of the same nucleotide length as the primer of SEQ ID NO: 29 and the second nucleotide may be of the same nucleotide length as the primer of SEQ ID NO: 30. The GC content of each of said first and second primers may independently be of 40-60%.

10 For example, for specific amplification from said gene vii. of SEQ ID NO: 6 (clone CC5), said primers may comprise

- the primer pair of SEQ ID NO: 32 and 33, or

- a primer pair, which still is specific of gene vii. of SEQ ID NO: 6 and which anneals to gene vii. of SEQ ID NO: 6 at positions that differ by ± 5 nucleotides (more particularly by ± 4 nucleotides, 15 by ± 3 nucleotides, by ± 2 nucleotides, by ± 1 nucleotides or by 0 nucleotide) from the positions at which the primer pair of SEQ ID NOs: 32 and 33 anneals to gene vii. of SEQ ID NO: 6; or

- a primer pair, which still is specific of gene vii. of SEQ ID NO: 6, and which anneals to gene vii. of SEQ ID NO: 6 to amplify

20 a fragment from gene vii. of SEQ ID NO: 6, wherein said fragment comprises the (target) sequence SEQ ID NO: 31 that is amplified from gene vii. of SEQ ID NO: 6 by the primer pair of SEQ ID NOs: 32 and 33 (*i.e.*, the target sequence of SEQ ID NO: 31) and is at most 10 nucleotides longer than said target sequence (more particularly at most 9, 8, 7, 6, 5, 4, 3, 2, 1 or 0 nucleotide(s) longer than said target sequence); or

25 a fragment from gene vii. of SEQ ID NO: 6, wherein said fragment consists of the (target) sequence that is amplified from gene vii. of SEQ ID NO: 6 by the primer pair of SEQ ID NOs: 32 and 33 (*i.e.*, the sequence of SEQ ID NO: 31) but deleted from at most 10 nucleotides (more particularly at most 9, 8, 7, 6, 5, 4, 3, 2, 1 or 0 nucleotide(s)); or

- a primer pair, which anneals to gene vii. of SEQ ID NO: 6 to specifically amplify (from said gene) the same target sequence as said primer pair of SEQ ID NOs: 32 and 33 (*i.e.*, the sequence 30 of SEQ ID NO: 31), wherein the first primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 32 and the second primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 33.

35 More particularly, for specific amplification from said gene vii. of SEQ ID NO: 6, said primers may comprise a primer pair for specifically amplifying (from said gene) the same target sequence as the primer pair of SEQ ID NOs: 32 and 33 (*i.e.*, the target sequence of SEQ ID NO: 31).

More particularly, for specific amplification from said gene vii. of SEQ ID NO: 6, said primers may comprise a primer pair for specifically amplifying (from said gene) the same target sequence as the primer pair of SEQ ID NOs: 32 and 33 (*i.e.*, the target sequence of SEQ ID NO: 31), wherein said primer pair is

- 5 - the primer pair of SEQ ID NOs: 32 and 33, or
 - a primer pair, which anneals to gene vii. of SEQ ID NO: 6 to specifically amplify (from said gene) the same target sequence as said primer pair of SEQ ID NOs: 32 and 33 (*i.e.*, the target sequence of SEQ ID NO: 31), wherein the first primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ
 - 10 ID NO: 32 and the second primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 33.
- Each of the first and second primers of the primer pair may independently consist of 15-30 nucleotides, more particularly 19-25 nucleotides, more particularly of 20-24 nucleotides, *i.e.*, of 20, 21, 22, 23 or 24 nucleotides. More particularly, the first nucleotide of the primer pair may be
- 15 of the same nucleotide length as the primer of SEQ ID NO: 32 and the second nucleotide may be of the same nucleotide length as the primer of SEQ ID NO: 33. The GC content of each of said first and second primers may independently be of 40-60%.

For example, for specific amplification from said gene v. of SEQ ID NO: 9 (clone CC7), said

- 20 primers may comprise
 - the primer pair of SEQ ID NO: 41 and 42, or
 - a primer pair, which still is specific of gene v. of SEQ ID NO: 9 and which anneals to gene v. of SEQ ID NO: 9 at positions that differ by ± 5 nucleotides (more particularly by ± 4 nucleotides, by ± 3 nucleotides, by ± 2 nucleotides, by ± 1 nucleotides or by 0 nucleotide) from the positions at
 - 25 which the primer pair of SEQ ID NOs: 41 and 42 anneals to gene v. of SEQ ID NO: 9; or
 - a primer pair, which still is specific of gene v. of SEQ ID NO: 9, and which anneals to gene v. of SEQ ID NO: 9 to amplify
- a fragment from gene v. of SEQ ID NO: 9, wherein said fragment comprises the (target) sequence SEQ ID NO: 40 that is amplified from gene v. of SEQ ID NO: 9 by the primer pair
 - 30 of SEQ ID NOs: 41 and 42 (*i.e.*, the target sequence of SEQ ID NO: 40) and is at most 10 nucleotides longer than said target sequence (more particularly at most 9, 8, 7, 6, 5, 4, 3, 2, 1 or 0 nucleotide(s) longer than said target sequence); or
 - a fragment from gene v. of SEQ ID NO: 9, wherein said fragment consists of the (target) sequence that is amplified from gene v. of SEQ ID NO: 9 by the primer pair of SEQ ID
 - 35 NOs: 41 and 42 (*i.e.*, the sequence of SEQ ID NO: 40) but deleted from at most 10 nucleotides (more particularly at most 9, 8, 7, 6, 5, 4, 3, 2, 1 or 0 nucleotide(s)); or

- a primer pair, which anneals to gene v. of SEQ ID NO: 9 to specifically amplify (from said gene) the same target sequence as said primer pair of SEQ ID NOs: 41 and 42 (*i.e.*, the sequence of SEQ ID NO: 40), wherein the first primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 41 and the second primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 42.
- More particularly, for specific amplification from said gene v. of SEQ ID NO: 9, said primers may comprise a primer pair for specifically amplifying (from said gene) the same target sequence as the primer pair of SEQ ID NOs: 41 and 42 (*i.e.*, the target sequence of SEQ ID NO: 40).
- More particularly, for specific amplification from said gene v. of SEQ ID NO: 9, said primers may comprise a primer pair for specifically amplifying (from said gene) the same target sequence as the primer pair of SEQ ID NOs: 41 and 42 (*i.e.*, the target sequence of SEQ ID NO: 40), wherein said primer pair is
- the primer pair of SEQ ID NOs: 41 and 42, or
 - a primer pair, which anneals to gene v. of SEQ ID NO: 9 to specifically amplify (from said gene) the same target sequence as said primer pair of SEQ ID NOs: 41 and 42 (*i.e.*, the target sequence of SEQ ID NO: 40), wherein the first primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 41 and the second primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 42.
- Each of the first and second primers of the primer pair may independently consist of 15-30 nucleotides, more particularly 19-25 nucleotides, more particularly of 20-24 nucleotides, *i.e.*, of 20, 21, 22, 23 or 24 nucleotides. More particularly, the first nucleotide of the primer pair may be of the same nucleotide length as the primer of SEQ ID NO: 41 and the second nucleotide may be of the same nucleotide length as the primer of SEQ ID NO: 42. The GC content of each of said first and second primers may independently be of 40-60%.
- For example, for specific amplification from said gene viii. of SEQ ID NO: 10 (clone CC8 and/or (and) CC16)), said primers may comprise
- the primer pair of SEQ ID NO: 44 and 45, or
 - a primer pair, which still is specific of gene viii. of SEQ ID NO: 10 and which anneals to gene viii. of SEQ ID NO: 10 at positions that differ by ± 5 nucleotides (more particularly by ± 4 nucleotides, by ± 3 nucleotides, by ± 2 nucleotides, by ± 1 nucleotides or by 0 nucleotide) from the positions at which the primer pair of SEQ ID NOs: 44 and 45 anneals to gene viii. of SEQ ID NO: 10; or
 - a primer pair, which still is specific of gene viii. of SEQ ID NO: 10, and which anneals to gene viii. of SEQ ID NO: 10 to amplify

- a fragment from gene viii. of SEQ ID NO: 10, wherein said fragment comprises the (target) sequence SEQ ID NO: 43 that is amplified from gene viii. of SEQ ID NO: 10 by the primer pair of SEQ ID NOs: 44 and 45 (*i.e.*, the target sequence of SEQ ID NO: 43) and is at most 10 nucleotides longer than said target sequence (more particularly at most 9, 8, 7, 6, 5, 4, 3, 2, 1 or 0 nucleotide(s) longer than said target sequence); or
- a fragment from gene viii. of SEQ ID NO: 10, wherein said fragment consists of the (target) sequence that is amplified from gene viii. of SEQ ID NO: 10 by the primer pair of SEQ ID NOs: 44 and 45 (*i.e.*, the sequence of SEQ ID NO: 43) but deleted from at most 10 nucleotides (more particularly at most 9, 8, 7, 6, 5, 4, 3, 2, 1 or 0 nucleotide(s)); or
- a primer pair, which anneals to gene viii. of SEQ ID NO: 10 to specifically amplify (from said gene) the same target sequence as said primer pair of SEQ ID NOs: 44 and 45 (*i.e.*, the sequence of SEQ ID NO: 43), wherein the first primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 44 and the second primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 45.
- More particularly, for specific amplification from said gene viii. of SEQ ID NO: 10, said primers may comprise a primer pair for specifically amplifying (from said gene) the same target sequence as the primer pair of SEQ ID NOs: 44 and 45 (*i.e.*, the target sequence of SEQ ID NO: 43).
- More particularly, for specific amplification from said gene viii. of SEQ ID NO: 10, said primers may comprise a primer pair for specifically amplifying (from said gene) the same target sequence as the primer pair of SEQ ID NOs: 44 and 45 (*i.e.*, the target sequence of SEQ ID NO: 43), wherein said primer pair is
- the primer pair of SEQ ID NOs: 44 and 45, or
 - a primer pair, which anneals to gene viii. of SEQ ID NO: 10 to specifically amplify (from said gene) the same target sequence as said primer pair of SEQ ID NOs: 44 and 45 (*i.e.*, the target sequence of SEQ ID NO: 43), wherein the first primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 44 and the second primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 45.
- Each of the first and second primers of the primer pair may independently consist of 15-30 nucleotides, more particularly 19-25 nucleotides, more particularly of 20-24 nucleotides, *i.e.*, of 20, 21, 22, 23 or 24 nucleotides. More particularly, the first nucleotide of the primer pair may be of the same nucleotide length as the primer of SEQ ID NO: 44 and the second nucleotide may be of the same nucleotide length as the primer of SEQ ID NO: 45. The GC content of each of said first and second primers may independently be of 40-60%.

For example, for specific amplification from said gene ix. of SEQ ID NO: 8 (clone CC155), said primers may comprise

- the primer pair of SEQ ID NO: 38 and 39, or
 - a primer pair, which still is specific of gene ix. of SEQ ID NO: 8 and which anneals to gene ix. of SEQ ID NO: 8 at positions that differ by ± 5 nucleotides (more particularly by ± 4 nucleotides, by ± 3 nucleotides, by ± 2 nucleotides, by ± 1 nucleotides or by 0 nucleotide) from the positions at which the primer pair of SEQ ID NOs: 38 and 39 anneals to gene ix. of SEQ ID NO: 8; or
 - a primer pair, which still is specific of gene ix. of SEQ ID NO: 8, and which anneals to gene ix. of SEQ ID NO: 8 to amplify
- 10 a fragment from gene ix. of SEQ ID NO: 8, wherein said fragment comprises the (target) sequence SEQ ID NO: 37 that is amplified from gene ix. of SEQ ID NO: 8 by the primer pair of SEQ ID NOs: 38 and 39 (*i.e.*, the target sequence of SEQ ID NO: 37) and is at most 10 nucleotides longer than said target sequence (more particularly at most 9, 8, 7, 6, 5, 4, 3, 2, 1 or 0 nucleotide(s) longer than said target sequence); or
- 15 a fragment from gene ix. of SEQ ID NO: 8, wherein said fragment consists of the (target) sequence that is amplified from gene ix. of SEQ ID NO: 8 by the primer pair of SEQ ID NOs: 38 and 39 (*i.e.*, the sequence of SEQ ID NO: 37) but deleted from at most 10 nucleotides (more particularly at most 9, 8, 7, 6, 5, 4, 3, 2, 1 or 0 nucleotide(s)); or
- a primer pair, which anneals to gene ix. of SEQ ID NO: 8 to specifically amplify (from said
- 20 gene) the same target sequence as said primer pair of SEQ ID NOs: 38 and 39 (*i.e.*, the sequence of SEQ ID NO: 37), wherein the first primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 38 and the second primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 39.
- 25 More particularly, for specific amplification from said gene ix. of SEQ ID NO: 8, said primers may comprise a primer pair for specifically amplifying (from said gene) the same target sequence as the primer pair of SEQ ID NOs: 38 and 39 (*i.e.*, the target sequence of SEQ ID NO: 37).
- More particularly, for specific amplification from said gene ix. of SEQ ID NO: 8, said primers may comprise a primer pair for specifically amplifying (from said gene) the same target sequence
- 30 as the primer pair of SEQ ID NOs: 38 and 39 (*i.e.*, the target sequence of SEQ ID NO: 37), wherein said primer pair is
- the primer pair of SEQ ID NOs: 38 and 39, or
 - a primer pair, which anneals to gene ix. of SEQ ID NO: 8 to specifically amplify (from said
- 35 gene) the same target sequence as said primer pair of SEQ ID NOs: 38 and 39 (*i.e.*, the target sequence of SEQ ID NO: 37), wherein the first primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ

ID NO: 38 and the second primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 39.

Each of the first and second primers of the primer pair may independently consist of 15-30 nucleotides, more particularly 19-25 nucleotides, more particularly of 20-24 nucleotides, *i.e.*, of 20, 21, 22, 23 or 24 nucleotides. More particularly, the first nucleotide of the primer pair may be of the same nucleotide length as the primer of SEQ ID NO: 38 and the second nucleotide may be of the same nucleotide length as the primer of SEQ ID NO: 39. The GC content of each of said first and second primers may independently be of 40-60%.

10 For example, for specific amplification from said gene x. of SEQ ID NO: 7 (clone CC121), said primers may comprise

- the primer pair of SEQ ID NO: 35 and 36, or

- a primer pair, which still is specific of gene x. of SEQ ID NO: 7 and which anneals to gene x. of SEQ ID NO: 7 at positions that differ by ± 5 nucleotides (more particularly by ± 4 nucleotides, by ± 3 nucleotides, by ± 2 nucleotides, by ± 1 nucleotides or by 0 nucleotide) from the positions at

- a primer pair, which still is specific of gene x. of SEQ ID NO: 7, and which anneals to gene x. of SEQ ID NO: 7 to amplify

- 20 a fragment from gene x. of SEQ ID NO: 7, wherein said fragment comprises the (target) sequence SEQ ID NO: 34 that is amplified from gene x. of SEQ ID NO: 7 by the primer pair of SEQ ID NOs: 35 and 36 (*i.e.*, the target sequence of SEQ ID NO: 34) and is at most 10 nucleotides longer than said target sequence (more particularly at most 9, 8, 7, 6, 5, 4, 3, 2, 1 or 0 nucleotide(s) longer than said target sequence); or

- 25 a fragment from gene x. of SEQ ID NO: 7, wherein said fragment consists of the (target) sequence that is amplified from gene x. of SEQ ID NO: 7 by the primer pair of SEQ ID NOs: 35 and 36 (*i.e.*, the sequence of SEQ ID NO: 34) but deleted from at most 10 nucleotides (more particularly at most 9, 8, 7, 6, 5, 4, 3, 2, 1 or 0 nucleotide(s)); or

- a primer pair, which anneals to gene x. of SEQ ID NO: 7 to specifically amplify (from said gene) the same target sequence as said primer pair of SEQ ID NOs: 35 and 36 (*i.e.*, the sequence of SEQ ID NO: 34), wherein the first primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 35 and the second primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 36.

35 More particularly, for specific amplification from said gene x. of SEQ ID NO: 7, said primers may comprise a primer pair for specifically amplifying (from said gene) the same target sequence as the primer pair of SEQ ID NOs: 35 and 36 (*i.e.*, the target sequence of SEQ ID NO: 34).

More particularly, for specific amplification from said gene x. of SEQ ID NO: 7, said primers may comprise a primer pair for specifically amplifying (from said gene) the same target sequence as the primer pair of SEQ ID NOs: 35 and 36 (*i.e.*, the target sequence of SEQ ID NO: 34), wherein said primer pair is

- 5 - the primer pair of SEQ ID NOs: 35 and 36, or
- a primer pair, which anneals to gene x. of SEQ ID NO: 7 to specifically amplify (from said gene) the same target sequence as said primer pair of SEQ ID NOs: 35 and 36 (*i.e.*, the target sequence of SEQ ID NO: 34), wherein the first primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 35
- 10 and the second primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 36.

Each of the first and second primers of the primer pair may independently consist of 15-30 nucleotides, more particularly 19-25 nucleotides, more particularly of 20-24 nucleotides, *i.e.*, of 20, 21, 22, 23 or 24 nucleotides. More particularly, the first nucleotide of the primer pair may be

- 15 of the same nucleotide length as the primer of SEQ ID NO: 35 and the second nucleotide may be of the same nucleotide length as the primer of SEQ ID NO: 36. The GC content of each of said first and second primers may independently be of 40-60%.

For example, for specific amplification from said gene xi. of SEQ ID NO: 11 (clone CC9), said

- 20 primers may comprise

- the primer pair of SEQ ID NO: 47 and 48, or
- a primer pair, which still is specific of gene xi. of SEQ ID NO: 11 and which anneals to gene xi. of SEQ ID NO: 11 at positions that differ by ± 5 nucleotides (more particularly by ± 4 nucleotides, by ± 3 nucleotides, by ± 2 nucleotides, by ± 1 nucleotides or by 0 nucleotide) from the positions at
- 25 which the primer pair of SEQ ID NOs: 47 and 48 anneals to gene xi. of SEQ ID NO: 11; or
- a primer pair, which still is specific of gene xi. of SEQ ID NO: 11, and which anneals to gene xi. of SEQ ID NO: 11 to amplify

- a fragment from gene xi. of SEQ ID NO: 11, wherein said fragment comprises the (target) sequence SEQ ID NO: 46 that is amplified from gene xi. of SEQ ID NO: 11 by the primer pair of SEQ ID NOs: 47 and 48 (*i.e.*, the target sequence of SEQ ID NO: 46) and is at most
 - 30 10 nucleotides longer than said target sequence (more particularly at most 9, 8, 7, 6, 5, 4, 3, 2, 1 or 0 nucleotide(s) longer than said target sequence); or

- a fragment from gene xi. of SEQ ID NO: 11, wherein said fragment consists of the (target) sequence that is amplified from gene xi. of SEQ ID NO: 11 by the primer pair of SEQ ID
 - 35 NOs: 47 and 48 (*i.e.*, the sequence of SEQ ID NO: 46) but deleted from at most 10 nucleotides (more particularly at most 9, 8, 7, 6, 5, 4, 3, 2, 1 or 0 nucleotide(s)); or

- a primer pair, which anneals to gene xi. of SEQ ID NO: 11 to specifically amplify (from said gene) the same target sequence as said primer pair of SEQ ID NOs: 47 and 48 (*i.e.*, the sequence of SEQ ID NO: 46), wherein the first primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 47 and the second primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 48.
- More particularly, for specific amplification from said gene xi. of SEQ ID NO: 11, said primers may comprise a primer pair for specifically amplifying (from said gene) the same target sequence as the primer pair of SEQ ID NOs: 47 and 48 (*i.e.*, the target sequence of SEQ ID NO: 46).
- More particularly, for specific amplification from said gene xi. of SEQ ID NO: 11, said primers may comprise a primer pair for specifically amplifying (from said gene) the same target sequence as the primer pair of SEQ ID NOs: 47 and 48 (*i.e.*, the target sequence of SEQ ID NO: 46), wherein said primer pair is
- the primer pair of SEQ ID NOs: 47 and 48, or
 - a primer pair, which anneals to gene xi. of SEQ ID NO: 11 to specifically amplify (from said gene) the same target sequence as said primer pair of SEQ ID NOs: 47 and 48 (*i.e.*, the target sequence of SEQ ID NO: 46), wherein the first primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 47 and the second primer of said primer pair is at least 95% (more particularly at least 96%, at least 97%, at least 98% or at least 99%) identical to the primer of SEQ ID NO: 48.
- Each of the first and second primers of the primer pair may independently consist of 15-30 nucleotides, more particularly 19-25 nucleotides, more particularly of 20-24 nucleotides, *i.e.*, of 20, 21, 22, 23 or 24 nucleotides. More particularly, the first nucleotide of the primer pair may be of the same nucleotide length as the primer of SEQ ID NO: 47 and the second nucleotide may be of the same nucleotide length as the primer of SEQ ID NO: 48. The GC content of each of said first and second primers may independently be of 40-60%.

- In addition to determining the presence or absence of a plurality of said genes, more particularly the presence or absence of a plurality of genes comprising said genes i.-iv. and/or said genes vi.-vii. and/or said genes v., viii. and ix., a method of the application may further comprise determining the presence or absence of control marker gene(s), which is(are)
- a control marker gene or a combination of control marker genes, which is specific of *L. monocytogenes* serogroup IVb, and/or
 - a control marker gene or a combination of control marker genes, which is specific of *L. monocytogenes* serogroup IIb, and/or
 - a control marker gene or a combination of control marker genes, which is specific of *L. monocytogenes* serogroups IIa and IIc or of *L. monocytogenes* lineage II.

The control marker gene, which is specific of *L. monocytogenes* serogroup IVb, may be the *L. monocytogenes* gene of SEQ ID NO: 15 or of any one of SEQ ID NOs: 180-181, more particularly the *L. monocytogenes* gene of SEQ ID NO: 15.

5

The combination of control marker genes, which is specific of *L. monocytogenes* serogroup IIb, may be the combination of

- a control marker gene, which is specific of *L. monocytogenes* lineage I, and of
- a control marker gene, which is specific of serogroup IIb and lineage II of *L. monocytogenes*.

10 The control marker gene, which is specific of *L. monocytogenes* lineage I, may be the *L. monocytogenes* gene of SEQ ID NO: 12 or of any one of SEQ ID NOs: 156-157, more particularly the *L. monocytogenes* gene of SEQ ID NO: 12.

The control marker gene, which is specific of serogroup IIb and lineage II of *L. monocytogenes*, may be the *L. monocytogenes* gene of SEQ ID NO: 14 or of any one of SEQ ID NOs: 172-179, more particularly the *L. monocytogenes* gene of SEQ ID NO: 14.

15

The control marker gene, which is specific of *L. monocytogenes* serogroups IIa and IIc or of *L. monocytogenes* lineage II, may be the *L. monocytogenes* gene of SEQ ID NO: 13 or of any one of SEQ ID NOs: 158-171, more particularly the *L. monocytogenes* gene of SEQ ID NO: 13.

20

Primers and/or probes can be used to determine the presence or absence of said control marker gene(s).

Primers to determine the presence or absence of said control marker gene(s) may comprise primers for determining the presence or absence of the control marker gene of SEQ ID NO: 15 (which is specific of *L. monocytogenes* serogroup IVb), wherein said primers for determining the presence or absence of the control marker gene of SEQ ID NO: 15 are a primer pair for specific amplification from the control marker gene of SEQ ID NO: 15, and wherein said primer pair is

25

- the primer pair of SEQ ID NOs: 59 and 60, or

30

- a primer pair, which anneals to the control marker gene of SEQ ID NO: 15 to specifically amplify the same target sequence as said primer pair of SEQ ID NOs: 59 and 60 (*i.e.*, the target sequence of SEQ ID NO: 58), wherein the first primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 59 and the second primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 60.

35

Primers to determine the presence or absence of said control marker gene(s) may comprise primers for determining the presence or absence of the control marker gene of SEQ ID NO: 12 (which is

specific of *L. monocytogenes* lineage I), wherein said primers for determining the presence or absence of the control marker gene of SEQ ID NO: 12 are a primer pair for specific amplification from the control marker gene of SEQ ID NO: 12, and wherein said primer pair is

- the primer pair of SEQ ID NOs: 50 and 51, or
 - 5 - a primer pair, which anneals to the control marker gene of SEQ ID NO: 12 to specifically amplify the same target sequence as said primer pair of SEQ ID NOs: 50 and 51 (*i.e.*, the target sequence of SEQ ID NO: 49), wherein the first primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 50 and the second primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 51.
- 10 Primers to determine the presence or absence of said control marker gene(s) may comprise primers for determining the presence or absence of the control marker gene of SEQ ID NO: 14 (which is specific of serogroup IIb and lineage II of *L. monocytogenes*), wherein said primers for determining the presence or absence of the control marker gene of SEQ ID NO: 14 are a primer pair for specific amplification from the control marker gene of SEQ ID NO: 14, and wherein said
- 15 primer pair is
- the primer pair of SEQ ID NOs: 56 and 57, or
 - a primer pair, which anneals to the control marker gene of SEQ ID NO: 14 to specifically amplify the same target sequence as said primer pair of SEQ ID NOs: 56 and 57 (*i.e.*, the target sequence of SEQ ID NO: 55), wherein the first primer of said primer pair is at least
 - 20 95% identical to the primer of SEQ ID NO: 56 and the second primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 57.

- Primers to determine the presence or absence of said control marker gene(s) may comprise primers for determining the presence or absence of the control marker gene of SEQ ID NO: 12 (which is specific of *L. monocytogenes* lineage I), wherein said primers for determining the presence or
- 25 absence of the control marker gene of SEQ ID NO: 12 are a primer pair for specific amplification from the control marker gene of SEQ ID NO: 12, and wherein said primer pair is

- the primer pair of SEQ ID NOs: 50 and 51, or
- a primer pair, which anneals to the control marker gene of SEQ ID NO: 12 to specifically amplify the same target sequence as said primer pair of SEQ ID NOs: 50 and 51 (*i.e.*, the
- 30 target sequence of SEQ ID NO: 49), wherein the first primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 50 and the second primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 51,

- and may further comprise primers for determining the presence or absence of the control marker gene of SEQ ID NO: 14 (which is specific of serogroup IIb and lineage II of *L. monocytogenes*),
- 35 wherein said primers for determining the presence or absence of the control marker gene of SEQ ID NO: 14 are a primer pair for specific amplification from the control marker gene of SEQ ID NO: 14, and wherein said primer pair is

- the primer pair of SEQ ID NOs: 56 and 57, or
- a primer pair, which anneals to the control marker gene of SEQ ID NO: 14 to specifically amplify the same target sequence as said primer pair of SEQ ID NOs: 56 and 57 (*i.e.*, the target sequence of SEQ ID NO: 55), wherein the first primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 56 and the second primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 57.

Primers to determine the presence or absence of said control marker gene(s) may comprise primers for determining the presence or absence of the control marker gene of SEQ ID NO: 13 (which is specific of *L. monocytogenes* serogroups IIa and IIc or of *L. monocytogenes* lineage II), wherein said primers for determining the presence or absence of the control marker gene of SEQ ID NO: 13 are a primer pair for specific amplification from the control marker gene of SEQ ID NO: 13, and wherein said primer pair is

- the primer pair of SEQ ID NOs: 53 and 54, or
- a primer pair, which anneals to the control marker gene of SEQ ID NO: 13 to specifically amplify the same target sequence as said primer pair of SEQ ID NOs: 53 and 54 (*i.e.*, the target sequence of SEQ ID NO: 52), wherein the first primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 53 and the second primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 54.

20

Advantageously, said primers for specific amplification from said gene i. of SEQ ID NO: 1, more particularly the primers of SEQ ID NOs: 17 and 18, can be used in mixture with said primers for specific amplification from said gene ii. of SEQ ID NO: 2, more particularly the primers of SEQ ID NOs: 20 and 21, with said primers for specific amplification from said gene iii. of SEQ ID NO: 3, more particularly the primers of SEQ ID NOs: 23 and 24, and with said primers for specific amplification from said gene iv. of SEQ ID NO: 4, more particularly the primers of SEQ ID NOs: 26 and 27, thereby forming a multiplex of oligonucleotides (comprising four primer pairs in mixture).

30 The application thus relates to a multiplex of oligonucleotides, which comprises at least two, at least three or the four of said primer pairs, thereby forming a multiplex of primers for specific amplification from at least two, at least three or the four of said genes i.-iv.. The application also relates to a container (such as a tube suitable for nucleic acid amplification, or such as a kit), which contains this multiplex of oligonucleotides or primers.

35 Advantageously, said (multiplex of) primers comprise

- a primer pair for specifically amplifying the sequence of SEQ ID NO: 16 from said gene i. of SEQ ID NO: 1,

- a primer pair for specifically amplifying the sequence of SEQ ID NO: 19 from said gene ii. of SEQ ID NO: 2,

- a primer pair for specifically amplifying the sequence of SEQ ID NO: 22 from said gene iii. of SEQ ID NO: 3; and

5 - a primer pair for specifically amplifying the sequence of SEQ ID NO: 25 from said gene iv. of SEQ ID NO: 4.

Each primer may independently consist of 15-30 nucleotides, more particularly 19-25 nucleotides, more particularly of 20-24 nucleotides, *i.e.*, of 20, 21, 22, 23 or 24 nucleotides

Advantageously, said (multiplex of) primers comprise

10 - a primer pair for specifically amplifying the sequence of SEQ ID NO: 16 from said gene i. of SEQ ID NO: 1, which is

- the primer pair of SEQ ID NOs: 17 and 18, or

- a primer pair, (which anneals to gene i. of SEQ ID NO: 1 to specifically amplify (from said gene) the sequence of SEQ ID NO: 16,) wherein the first primer of said primer pair is at least
15 95% identical to the primer of SEQ ID NO: 17 and is of the same nucleotide length as SEQ ID NO: 17, and wherein the second primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 18 and is of the same nucleotide length as SEQ ID NO: 18;

- a primer pair for specifically amplifying the sequence of SEQ ID NO: 19 from said gene ii. of SEQ ID NO: 2, which is

20 - the primer pair of SEQ ID NOs: 20 and 21, or

- a primer pair, (which anneals to gene ii. of SEQ ID NO: 2 to specifically amplify (from said gene) the sequence of SEQ ID NO: 19,) wherein the first primer of said primer pair is at least
25 95% identical to the primer of SEQ ID NO: 20 and is of the same nucleotide length as SEQ ID NO: 20, and wherein the second primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 21 and is of the same nucleotide length as SEQ ID NO: 21;

- a primer pair for specifically amplifying the sequence of SEQ ID NO: 22 from said gene iii. of SEQ ID NO: 3, which is

- the primer pair of SEQ ID NOs: 23 and 24, or

- a primer pair, (which anneals to gene iii. of SEQ ID NO: 3 to specifically amplify (from said gene) the sequence of SEQ ID NO: 22,) wherein the first primer of said primer pair is at least
30 95% identical to the primer of SEQ ID NO: 23 and is of the same nucleotide length as SEQ ID NO: 23, and wherein the second primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 24 and is of the same nucleotide length as SEQ ID NO: 24; and

- a primer pair for specifically amplifying the sequence of SEQ ID NO: 25 from said gene iv. of
35 SEQ ID NO: 4, which is

- the primer pair of SEQ ID NOs: 26 and 27, or

- a primer pair, (which anneals to gene iv. of SEQ ID NO: 4 to specifically amplify (from said gene) the sequence of SEQ ID NO: 25,) wherein the first primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 26 and is of the same nucleotide length as SEQ ID NO: 26, and wherein the second primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 27 and is of the same nucleotide length as SEQ ID NO: 27.

An example of such a multiplex of primers is shown in the examples below (multiplex IVb of the application).

- Advantageously, said primers for specific amplification from said gene vi. of SEQ ID NO: 5, more particularly the primers of SEQ ID NOs: 29 and 30, can be used in mixture with said primers for specific amplification from said gene vii. of SEQ ID NO: 6, more particularly the primers of SEQ ID NOs: 32 and 33, thereby forming a multiplex of oligonucleotides (comprising said two primer pairs in mixture).

- The application thus relates to a multiplex of oligonucleotides, which comprises the two of said primer pairs, thereby forming a multiplex of primers for specific amplification from each of said genes vi. and vii. The application also relates to a container (such as a tube suitable for nucleic acid amplification, or such as a kit), which contains this multiplex.

Advantageously, said (multiplex of) primers comprise

- a primer pair for specifically amplifying the sequence of SEQ ID NO: 28 from said gene vi. of SEQ ID NO: 5; and
- a primer pair for specifically amplifying the sequence of SEQ ID NO: 31 from said gene vii. of SEQ ID NO: 6.

Each primer may independently consist of 15-30 nucleotides, more particularly 19-25 nucleotides, more particularly of 20-24 nucleotides, *i.e.*, of 20, 21, 22, 23 or 24 nucleotides

- Advantageously, said (multiplex of) primers comprise

- a primer pair for specifically amplifying the sequence of SEQ ID NO: 28 from said gene vi. of SEQ ID NO: 5, which is
 - the primer pair of SEQ ID NOs: 29 and 30, or
 - a primer pair, (which anneals to gene vi. of SEQ ID NO: 5 to specifically amplify (from said gene) the sequence of SEQ ID NO: 28), wherein the first primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 29 and is of the same nucleotide length as SEQ ID NO: 29, and wherein the second primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 30 and is of the same nucleotide length as SEQ ID NO: 30; and
- a primer pair for specifically amplifying the sequence of SEQ ID NO: 31 from said gene vii. of SEQ ID NO: 6, which is
 - the primer pair of SEQ ID NOs: 32 and 33, or

- a primer pair, (which anneals to gene vii. of SEQ ID NO: 6 to specifically amplify (from said gene) the sequence of SEQ ID NO: 31), wherein the first primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 32 and is of the same nucleotide length as SEQ ID NO: 32, and wherein the second primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 33 and is of the same nucleotide length as SEQ ID NO: 33.

An example of such a multiplex of primers is shown in the examples below (multiplex IIB of the application).

- Advantageously, said primers for specific amplification from said gene v. of SEQ ID NO: 9, more particularly the primers of SEQ ID NOs: 41 and 42, can be used in mixture with said primers for specific amplification from said gene viii. of SEQ ID NO: 10, more particularly the primers of SEQ ID NOs: 44 and 45, with said primers for specific amplification from said gene ix. of SEQ ID NO: 8, more particularly the primers of SEQ ID NOs: 38 and 39, and thereby forming a multiplex of oligonucleotides (comprising said three primer pairs in mixture).

- The application thus relates to a multiplex of oligonucleotides, which comprises at least two or the three of said primer pairs, thereby forming a multiplex of primers for specific amplification from at least two or the three of said genes v., viii. and ix. The application also relates to a container (such as a tube suitable for nucleic acid amplification, or such as a kit), which contains this multiplex of primers.

Advantageously, said (multiplex of) primers comprise

- a primer pair for specifically amplifying the sequence of SEQ ID NO: 40 from said gene v. of SEQ ID NO: 9;
- a primer pair for specifically amplifying the sequence of SEQ ID NO: 43 from said gene viii. of SEQ ID NO: 10; and
- a primer pair for specifically amplifying the sequence of SEQ ID NO: 37 from said gene ix. of SEQ ID NO: 8.

Each primer may independently consist of 15-30 nucleotides, more particularly 19-25 nucleotides, more particularly of 20-24 nucleotides, *i.e.*, of 20, 21, 22, 23 or 24 nucleotides

- Advantageously, said (multiplex of) primers comprise

- a primer pair for specifically amplifying the sequence of SEQ ID NO: 40 from said gene v. of SEQ ID NO: 9, which is
 - the primer pair of SEQ ID NOs: 41 and 42, or
 - a primer pair, (which anneals to gene v. of SEQ ID NO: 9 to specifically amplify (from said gene) the sequence of SEQ ID NO: 40,) wherein the first primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 41 and is of the same nucleotide length as SEQ

ID NO: 41, and wherein the second primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 42 and is of the same nucleotide length as SEQ ID NO: 42;

- a primer pair for specifically amplifying the sequence of SEQ ID NO: 43 from said gene viii. of SEQ ID NO: 10, which is

5 - the primer pair of SEQ ID NOs: 44 and 45, or

- a primer pair, (which anneals to gene viii. of SEQ ID NO: 10 to specifically amplify (from said gene) the sequence of SEQ ID NO: 43,) wherein the first primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 44 and is of the same nucleotide length as SEQ ID NO: 44, and wherein the second primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 45 and is of the same nucleotide length as SEQ ID NO: 45; and

10 - a primer pair for specifically amplifying the sequence of SEQ ID NO: 37 from said gene ix. of SEQ ID NO: 8, which is

- the primer pair of SEQ ID NOs: 38 and 39, or

15 - a primer pair, (which anneals to gene ix. of SEQ ID NO: 8 to specifically amplify (from said gene) the sequence of SEQ ID NO: 37,) wherein the first primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 38 and is of the same nucleotide length as SEQ ID NO: 38, and wherein the second primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 39 and is of the same nucleotide length as SEQ ID NO: 39.

Said (multiplex of) primers may further comprise

20 - a primer pair suitable for nucleic acid amplification from gene x., *i.e.*, from a gene, which is specific of *L. monocytogenes* clone CC121, wherein said CC121-specific gene is *LM6179_0610* (SEQ ID NO: 7) or one of the genes of SEQ ID NOs: 123-128, more particularly *LM6179_0610* (SEQ ID NO: 7) (*cf.* above for the description of primers for specific amplification from said gene x. of SEQ ID NO: 7 (clone CC121)), and/or

25 - a primer pair suitable for nucleic acid amplification from gene xi., *i.e.*, from a gene, which is specific of *L. monocytogenes* clone CC9, wherein said CC9-specific gene is *lmo1118* (SEQ ID NO: 11)) (*cf.* above for the description of primers for specific amplification from said gene xi. of SEQ ID NO: 11 (clone CC9)),

30 thereby forming a multiplex of 4 or 5 primer pairs suitable for (specific) nucleic acid amplification from genes v., viii., ix. and from genes x. and/or xi.

An example of such a multiplex of primers is shown in the examples below (multiplex IIaIIc of the application).

Each of the three of the above-mentioned multiplex of oligonucleotides, *i.e.*

35 - the multiplex of oligonucleotides comprising primers for specific amplification from at least two, at least three or the four of said genes i.-iv.,

- the multiplex of oligonucleotides comprising for specific amplification from the two of said genes vi.-vii., and

- the multiplex of oligonucleotides comprising for specific amplification from at least two or the three of said genes v., viii., ix. (and optionally from one or the two of genes x. and/or xi.),
 5 may further comprise at least one (*e.g.*, one or two) primer pair for specific amplification from a control marker gene or a combination of (*e.g.*, two) control marker genes.

For example, the multiplex of primers for specific amplification from at least two, at least three or the four of said genes i.-iv. [multiplex IVb in the examples below] may further comprise a primer
 10 pair for specific amplification from a control marker gene, which is specific of *L. monocytogenes* serogroup IVb.

Said control marker gene may be the *L. monocytogenes* gene of SEQ ID NO: 15 or of any one of SEQ ID NOs: 180-181, more particularly the *L. monocytogenes* gene of SEQ ID NO: 15.

A primer pair for specific amplification from the control marker gene of SEQ ID NO: 15 may *e.g.*,
 15 be:

- the primer pair of SEQ ID NOs: 59 and 60, or
- a primer pair, which anneals to the control marker gene of SEQ ID NO: 15 to specifically amplify the same target sequence as said primer pair of SEQ ID NOs: 59 and 60 (*i.e.*, the target sequence of SEQ ID NO: 58), wherein the first primer of said primer pair is at least
 20 95% identical to the primer of SEQ ID NO: 59 and the second primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 60.

For example, the multiplex of primers for specific amplification from the two of said genes vi.-vii. [multiplex IIb in the examples below] may further comprise primer pairs for specific amplification
 25 from a combination of two control marker genes, wherein the first of said control marker gene is specific of *L. monocytogenes* lineage I and the second of said control marker gene is specific of serogroup IIb and lineage II of *L. monocytogenes*.

Said combination of control marker genes may comprise

- the *L. monocytogenes* gene of SEQ ID NO: 12 or of any one of SEQ ID NOs: 156-157 (which is
 30 specific of *L. monocytogenes* lineage I), more particularly the *L. monocytogenes* gene of SEQ ID NO: 12, and
- the *L. monocytogenes* gene of SEQ ID NO: 14 or of any one of SEQ ID NOs: 172-179 (which is specific of serogroup IIb and lineage II of *L. monocytogenes*), more particularly the *L. monocytogenes* gene of SEQ ID NO: 14.

35 Primer pairs for specific amplification from the combination of the control marker gene of SEQ ID NOs: 12 and 14 may *e.g.*, be:

- a first primer pair, which is

- the primer pair of SEQ ID NOs: 50 and 51, or
 - a primer pair, which anneals to the control marker gene of SEQ ID NO: 12 to specifically amplify the same target sequence as said primer pair of SEQ ID NOs: 50 and 51 (*i.e.*, the target sequence of SEQ ID NO: 49), wherein the first primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 50 and the second primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 51,
- and
- a second primer pair, which is
 - the primer pair of SEQ ID NOs: 56 and 57, or
 - a primer pair, which anneals to the control marker gene of SEQ ID NO: 14 to specifically amplify the same target sequence as said primer pair of SEQ ID NOs: 56 and 57 (*i.e.*, the target sequence of SEQ ID NO: 55), wherein the first primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 56 and the second primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 57.

15

For example, the multiplex of the multiplex of primers for specific amplification from at least two or the three of said genes v., viii., ix. (and optionally from one or the two of genes x. and/or xi.) [multiplex IIaIIc in the examples below] may further comprise a primer pair for specific amplification from a control marker gene, which is specific of *L. monocytogenes* serogroups IIa and IIc or of *L. monocytogenes* lineage II.

20

Said control marker gene may be the *L. monocytogenes* gene of SEQ ID NO: 13 or of any one of SEQ ID NOs: 158-171, more particularly the *L. monocytogenes* gene of SEQ ID NO: 13.

A primer pair for specific amplification from the control marker gene of SEQ ID NO: 13 may *e.g.*, be:

25

- the primer pair of SEQ ID NOs: 53 and 54, or
- a primer pair, which anneals to the control marker gene of SEQ ID NO: 13 to specifically amplify the same target sequence as said primer pair of SEQ ID NOs: 53 and 54 (*i.e.*, the target sequence of SEQ ID NO: 52), wherein the first primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 53 and the second primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 54.

30

Advantageously, the three of the above-mentioned multiplex of primers, *i.e.*,

35

- the multiplex of oligonucleotides comprising primers for specific amplification from at least two, at least three or the four of said genes i.-iv. (and optionally from the control marker gene that is specific of serogroup IVb),
- the multiplex of oligonucleotides comprising primers for specific amplification from the two of said genes vi.-vii. (and optionally from the combination of a control marker gene that is

specific of lineage I and of a control marker gene that is specific of serogroup IIb and lineage II), and

- the multiplex of oligonucleotides comprising primers for specific amplification from at least two or the three of said genes v., viii., ix. and optionally from one or the two of genes x. and/or xi. (and optionally from the control marker gene that is specific of serogroups IIa and IIc, or of lineage II),

can be implemented under the same DNA amplification thermocycling conditions, more particularly under the same PCR thermocycling conditions. The three of the above-mentioned multiplex of primers can thus be implemented simultaneously on the same DNA amplification plate, more particularly on the same PCR plate.

Examples of such PCR thermocycling conditions include an (initial) denaturation step at 94°C for 30 s followed by 25 cycles of 30 s at 94°C, 30 s at 55°C and 75 s at 72°C, and a (final) elongation step at 72°C for 10 min (*cf.* the examples below).

- 15 The primers of the application may thus comprise:

- the multiplex of primers for specific amplification from at least two, at least three or the four of said genes i.-iv. (and optionally from the control marker gene that is specific of serogroup IVb), and

- the multiplex of primers for specific amplification from the two of said genes vi.-vii. (and optionally from the combination of a control marker gene that is specific of lineage I and of a control marker gene that is specific of serogroup IIb and lineage II).

Said primers can be implemented simultaneously under the same DNA amplification (more particularly PCR) thermocycling conditions.

- Examples of such PCR thermocycling conditions include an (initial) denaturation step at 94°C for 30 s followed by 25 cycles of 30 s at 94°C, 30 s at 55°C and 75 s at 72°C, and a (final) elongation step at 72°C for 10 min (*cf.* the examples below).

The primers of the application may thus comprise:

- the multiplex of primers for specific amplification from the two of said genes vi.-vii. (and optionally from the combination of a control marker gene that is specific of lineage I and of a control marker gene that is specific of serogroup IIb and lineage II), and

- the multiplex of primers for specific amplification from at least two or the three of said genes v., viii., ix. and optionally from one or the two of genes x. and/or xi. (and optionally from the control marker gene that is specific of serogroups IIa and IIc, or of lineage II).

- Said primers can be implemented simultaneously under the same DNA amplification (more particularly PCR) thermocycling conditions.

Examples of such PCR thermocycling conditions include an (initial) denaturation step at 94°C for 30 s followed by 25 cycles of 30 s at 94°C, 30 s at 55°C and 75 s at 72°C, and a (final) elongation step at 72°C for 10 min (*cf.* the examples below).

5 The primers of the application may thus comprise:

- the multiplex of primers for specific amplification from at least two, at least three or the four of said genes i.-iv. (and optionally from the control marker gene that is specific of serogroup IVb), and

- the multiplex of primers for specific amplification from at least two or the three of said
10 genes v., viii., ix. and optionally from one or the two of genes x. and/or xi. (and optionally from the control marker gene that is specific of serogroups IIa and IIc, or of lineage II).

Said primers can be implemented simultaneously under the same DNA amplification (more particularly PCR) thermocycling conditions.

Examples of such PCR thermocycling conditions include an (initial) denaturation step at 94°C for
15 30 s followed by 25 cycles of 30 s at 94°C, 30 s at 55°C and 75 s at 72°C, and a (final) elongation step at 72°C for 10 min (*cf.* the examples below).

The primers of the application may thus comprise:

- the multiplex of primers for specific amplification from at least two, at least three or the
20 four of said genes i.-iv. (and optionally from the control marker gene that is specific of serogroup IVb),

- the multiplex of primers for specific amplification from the two of said genes vi.-vii. (and optionally from the combination of a control marker gene that is specific of lineage I and of a control marker gene that is specific of serogroup IIb and lineage II), and

- the multiplex of primers for specific amplification from at least two or the three of said
25 genes v., viii., ix. and optionally from one or the two of genes x. and/or xi. (and optionally from the control marker gene that is specific of serogroups IIa and IIc, or of lineage II).

Said primers can be implemented simultaneously under the same DNA amplification (more particularly PCR) thermocycling conditions.

30 Examples of such PCR thermocycling conditions include an (initial) denaturation step at 94°C for 30 s followed by 25 cycles of 30 s at 94°C, 30 s at 55°C and 75 s at 72°C, and a (final) elongation step at 72°C for 10 min (*cf.* the examples below).

In accordance with the application, one or several probes can be implemented alternatively to or
35 complementarily to said primers or to said multiplex of oligonucleotides or primers.

Said one or several probes may comprise at least one probe suitable for nucleic acid hybridization on one of genes i.-ix., i.-xi., i.-x., i.-ix. and xi., more particularly at least one probe suitable for nucleic acid hybridization on one of genes i.-ix., more particularly for at least one probe suitable for nucleic acid hybridization on one of genes i.-iv., or one of genes vi. and vii., or one of genes v., viii. and ix.

Advantageously, said genes i.-ix. are the genes of SEQ ID NOs: 1-6, 9, 10 and 8, respectively.

Advantageously, said genes x. and xi. are the genes of SEQ ID NOs: 7 and 11, respectively.

Advantageously, said at least one probe, which hybridizes to genes i., hybridizes to a (target) sequence, which is the sequence of SEQ ID NO: 16 or the complementary sequence sequence thereof.

Advantageously, said at least one probe, which hybridizes to genes ii., hybridizes to a (target) sequence, which is the sequence of SEQ ID NO: 19 or the complementary sequence sequence thereof.

Advantageously, said at least one probe, which hybridizes to genes iii., hybridizes to a (target) sequence, which is the sequence of SEQ ID NO: 22 or the complementary sequence sequence thereof.

Advantageously, said at least one probe, which hybridizes to genes iv., hybridizes to a (target) sequence, which is the sequence of SEQ ID NO: 25 or the complementary sequence sequence thereof.

Advantageously, said at least one probe, which hybridizes to genes vi., hybridizes to a (target) sequence, which is the sequence of SEQ ID NO: 28 or the complementary sequence sequence thereof.

Advantageously, said at least one probe, which hybridizes to genes vii., hybridizes to a (target) sequence, which is the sequence of SEQ ID NO: 31 or the complementary sequence sequence thereof.

Advantageously, said at least one probe, which hybridizes to genes v., hybridizes to a (target) sequence, which is the sequence of SEQ ID NO: 40 or the complementary sequence sequence thereof.

Advantageously, said at least one probe, which hybridizes to genes viii., hybridizes to a (target) sequence, which is the sequence of SEQ ID NO: 43 or the complementary sequence sequence thereof.

Advantageously, said at least one probe, which hybridizes to genes ix., hybridizes to a (target) sequence, which is the sequence of SEQ ID NO: 37 or the complementary sequence sequence thereof.

Advantageously, said at least one probe, which hybridizes to genes x., hybridizes to a (target) sequence, which is the sequence of SEQ ID NO: 34 or the complementary sequence sequence thereof.

Advantageously, said at least one probe, which hybridizes to genes xi., hybridizes to a (target) sequence, which is the sequence of SEQ ID NO: 46 or the complementary sequence thereof.

- 5 The sequence of a probe is such that the presence of hybridization is indicative that said gene is present and, conversely, the absence of hybridization is indicative that said gene is absent.
Advantageously, said at least one probe is a probe, which specifically hybridizes to said gene or target sequence.

- 10 The sequence of said probe may comprise or consist of a hybridization sequence of 15-60 nucleotides (more particularly of 20-55 nucleotides, more particularly of 30-50 nucleotides, more particularly of 35-45 nucleotides). The hybridization sequence may be covalently linked to other nucleotide sequence(s) (*e.g.*, directly at the 5' end and/or at the 3' end of the hybridization sequence), wherein these other nucleotide sequence(s) do not (directly) contribute to, or improve,
15 the hybridization of the probe to the gene or target sequence, but which contribute to the detection of the probe, such as *e.g.*, beacon arm(s). These other nucleotide sequence may *e.g.*, consist of a total of 5-10 nucleotides (more particularly of 5, 6, 7, 8, 9 or 10 nucleotides).

- The sequence of said at least one probe may thus consist of a total of 25-70 nucleotides (more particularly of 25-65 nucleotides, more particularly 35-60 nucleotides, more particularly of 40-55
20 nucleotides).

- Said hybridization sequence may *e.g.*, be at least 90% identical (more particularly at least 91% or at least 92% or at least 93% identical or at least 94% identical or at least 95% identical or at least 96% or at least 97% or at least 98% or at least 99% or 100% identical) to the sequence that is complementary to a fragment of the gene or target sequence to which said probe (specifically)
25 hybridizes, wherein said fragment is of the same length as said hybridization sequence. Said fragment advantageously is internal to said gene or target sequence *e.g.*, said fragment does not comprise the first 10 nucleotides and the last 10 nucleotides at the 5' and 3' ends respectively of said gene or target sequence.

- 30 The application more particularly encompasses the implementation of several of such probes, as well as a set comprising several of such probes.

- Said probes may comprise several probes, wherein each probe is suitable for nucleic acid hybridization on different genes, said different genes being selected from genes i.-ix., i.-xi., i.-x. or i.-ix. and xi., more particularly from genes i.-ix., more particularly from genes i.-iv. and/or from
35 genes vi.-vii. and/or from genes v., viii. and ix.

Said probes may comprise at least two probes suitable for nucleic acid hybridization on one of genes i.-ix., more particularly at least one first probe suitable for nucleic acid hybridization on one

of genes i.-ix. and at least one second probe suitable for nucleic acid hybridization on another of genes i.-ix.

More particularly, said probes may comprise several probes, wherein each probe is suitable for nucleic acid hybridization on different genes, said different genes being each one of genes i.-iv.

5 and/or each one of genes vi.-vii. and/or each one of genes v., viii. and ix.

More particularly, said probes may comprise several probes, wherein each probe is suitable for nucleic acid hybridization on different genes, said different genes being each one of genes i.-ix.

The application more particularly relates to a set of probes comprising

- 10 - probes, which specifically hybridize to each one of genes i.-iv., and/or
- probes, which specifically hybridize to each one genes vi. and vii., and/or
- probes, which specifically hybridize to each one of genes v., viii. and ix.

Advantageously, said probes, which specifically hybridize to each one of genes i.-iv., specifically hybridizes to the (target) sequence of SEQ ID NO: 16 or to the complementary sequence thereof,
15 to the (target) sequence of SEQ ID NO: 19 or the complementary sequence thereof, to the (target) sequence of SEQ ID NO: 22 or the complementary sequence thereof, and to the (target) sequence of SEQ ID NO: 25 or the complementary sequence thereof.

Advantageously, said probes, which specifically hybridize to each one genes vi. and vii., specifically hybridizes to the (target) sequence of SEQ ID NO: 28 or the complementary sequence thereof and to the (target) sequence of SEQ ID NO: 31 or the complementary sequence thereof.

Advantageously, said probes, which specifically hybridize to each one of genes v., viii. and ix., specifically hybridizes to the (target) sequence of SEQ ID NO: 40 or the complementary sequence thereof, to the (target) sequence of SEQ ID NO: 43 or the complementary sequence thereof, and to the (target) sequence of SEQ ID NO: 37 or the complementary sequence thereof.

25

For example, said probes may comprise:

- a probe for specific hybridization to said gene i. of SEQ ID NO: 1, wherein said probe specifically hybridizes to SEQ ID NO: 1, more particularly to SEQ ID NO: 16, and wherein the sequence of said probe consists of 25-70 nucleotides;
- 30 - a probe for specific hybridization to said gene ii. of SEQ ID NO: 2, wherein said probe specifically hybridizes to SEQ ID NO: 2, more particularly to SEQ ID NO: 19, and wherein the sequence of said probe consists of 25-70 nucleotides;
- a probe for specific hybridization to said gene iii. of SEQ ID NO: 3, wherein said probe specifically hybridizes to SEQ ID NO: 3, more particularly to SEQ ID NO: 22, and wherein the
35 sequence of said probe consists of 25-70 nucleotides; and

- a probe for specific hybridization to said gene iv. of SEQ ID NO: 4, wherein said probe specifically hybridizes to SEQ ID NO: 4, more particularly to SEQ ID NO: 25, and wherein the sequence of said probe consists of 25-70 nucleotides.

For example, said probes may comprise:

- 5 - a probe for specific hybridization to said gene vi. of SEQ ID NO: 5, wherein said probe specifically hybridizes to SEQ ID NO: 5, more particularly to SEQ ID NO: 28, and wherein the sequence of said probe consists of 25-70 nucleotides; and
- a probe for specific hybridization to said gene vii. of SEQ ID NO: 6, wherein said probe specifically hybridizes to SEQ ID NO: 6, more particularly to SEQ ID NO: 31, and wherein the
- 10 sequence of said probe consists of 25-70 nucleotides.

For example, said probes may comprise:

- a probe for specific hybridization to said gene v. of SEQ ID NO: 9, wherein said probe specifically hybridizes to SEQ ID NO: 9, more particularly to SEQ ID NO: 40, and wherein the sequence of said probe consists of 25-70 nucleotides;
- 15 - a probe for specific hybridization to said gene viii. of SEQ ID NO: 10, wherein said probe specifically hybridizes to SEQ ID NO: 10, more particularly to SEQ ID NO: 43, and wherein the sequence of said probe consists of 25-70 nucleotides; and
- a probe for specific hybridization to said gene ix. of SEQ ID NO: 8, wherein said probe specifically hybridizes to SEQ ID NO: 8, more particularly to SEQ ID NO: 37, and wherein the
- 20 sequence of said probe consists of 25-70 nucleotides.

Advantageously, said probe for specific hybridization on gene i. of SEQ ID NO: 1 can be in fluidic contact (*e.g.*, on a solid support or array, such as a micro- or nano-array)

- with said probe for specific hybridization on gene ii. of SEQ ID NO: 2,
- 25 with said probe for specific hybridization on gene iii. of SEQ ID NO: 3, and
- with said probe for specific hybridization on gene iv. of SEQ ID NO: 4.

The application relates to a solid support or array, such as a micro- or nano-array, which comprises at least one, at least two, at least three or the four of said probes (covalently) linked thereto.

- Advantageously, said probe for specific hybridization on gene vi. of SEQ ID NO: 5 can be in
- 30 fluidic contact (*e.g.*, on a solid support or array, such as a micro- or nano-array) with said probe for specific hybridization on gene vii. of SEQ ID NO: 6,

The application relates to a solid support or array, such as a micro- or nano-array, which comprises at least one or the two of said probes (covalently) linked thereto.

- Advantageously, said probe for specific hybridization on gene v. of SEQ ID NO: 9 can be in
- 35 fluidic contact (*e.g.*, on a solid support or array, such as a micro- or nano-array)
- with said probe for specific hybridization on gene viii. of SEQ ID NO: 10, and
- with said probe for specific hybridization on gene ix. of SEQ ID NO: 8.

The application relates to a solid support or array, such as a micro- or nano-array, which comprises at least one, at least two or the three of said probes (covalently) linked thereto.

Advantageously, said probe for specific hybridization on gene x. of SEQ ID NO: 7 and/or said probe for specific hybridization on said gene xi. of SEQ ID NO: 11 can be in fluidic contact (*e.g.*,

5 on a solid support or array, such as a micro- or nano-array)

with said probe for specific hybridization on gene v. of SEQ ID NO: 9,

with said probe for specific hybridization on gene viii. of SEQ ID NO: 10, and

with said probe for specific hybridization on gene ix. of SEQ ID NO: 8.

10 The application relates to a solid support or array, such as a micro- or nano-array, which comprises at least one, at least two, at least three of the four of said probes (covalently) linked thereto.

Said probes may further comprise at least one probe, which specifically hybridizes to said serogroup IVb marker gene, more particularly to any one of SEQ ID NOs: 15 and 180-181, more particularly to said serogroup IVb marker gene of SEQ ID NO: 15.

15 Said probes may further comprise at least one probe, which specifically hybridizes to said lineage I marker gene, more particularly to any one of SEQ ID NOs: 12 and 156-157, more particularly to said lineage I marker gene of SEQ ID NO: 12, and at least one probe, which specifically hybridizes to said serogroup IIb and lineage II marker gene, more particularly to any one of SEQ ID NOs: 14 and 172-179, more particularly to said serogroup IIb and lineage II marker gene of
20 SEQ ID NO: 14.

Said probes may further comprise at least one probe, which specifically hybridizes to said lineage II marker gene, more particularly to any one of SEQ ID NOs: 13 and 158-171, more particularly to said lineage II marker gene of SEQ ID NO: 13.

25 A probe may further comprise at least one detection label, for example at least one fluorophore (*e.g.*, 6-carboxyfluorescein, or tetrachlorofluorescein).

Various formats (types) of probes, including TAQMAN™ probes (hydrolysis probes), beacon probes (*cf. e.g.*, Tyagi S. and Kramer F.R. 1996. Nat. Biotech. 14:303-308) and SCORPION™ probes (*cf. e.g.*, Whitcomb *et al.*, 1999, Nat. Biotech. 17:804-807) and the adjacent hybridization
30 probes (*cf. e.g.*, Wittwer, CT. *et al.*, 1997, BioTechniques 22:130-138), are known in the art.

In addition to the fluorophore, a probe may be covalently linked to a quencher (*e.g.*, a carboxytetramethylrhodamine fluorescent dye).

In addition to the hybridization sequence, the probe may comprise beacon arms, more particularly a 5' beacon arm and a 3' beacon arm, which impart a hairpin-configuration to the probe when
35 unhybridized (*e.g.*, the 3' beacon arm is complementary to the 5' beacon arm). A beacon arm may *e.g.*, consist of 5-10 nucleotides, more particularly of 5, 6, 7, 8, 9 or 10 nucleotides. Hence, a probe

in beacon format typically consists of 25-70 nucleotides, more particularly of 25-65 nucleotides, more particularly 35-60 nucleotides, more particularly of 40-55 nucleotides.

When used in real-time with a primer pair, the T_m of the probe may be 4-10°C higher than the T_m of the primer pair.

5

Primers and/or probes can be in mixture (*i.e.*, mixed together), *e.g.*, a multiplex of oligonucleotides comprising primers, or probes, or primers and probes, can be contained in the same amplification tube.

For example, the primers, or the primers and probes, for the determination the presence or absence of each of said genes i.-iv., can be contained in mixture in a first container, *e.g.*, a first tube suitable for nucleic acid amplification.

For example, the primers, or the primers and probes, for the determination the presence or absence of each of said genes vi.-vii. can be contained in mixture in a second container, *e.g.*, a second tube suitable for nucleic acid amplification.

For example, the primers, or the primers and probes, for the determination the presence or absence of each of said genes v., viii. and ix. can be contained in mixture in a third container, *e.g.*, a third tube suitable for nucleic acid amplification.

Alternatively, said primers, or probes, or primers and probes can be separate (*i.e.*, not mixed together) but contained in the same container. More particularly, they can be in fluidic contact, *i.e.*, without any material or physical barrier that would prevent a single drop of fluid (or of liquid) to contact all the probes (*e.g.*, probes, which are attached at separate locations in the same spot of a micro- or nano-array).

More particularly, said primers are in mixture in a container (such as a tube suitable for nucleic acid amplification, or such as a kit).

More particularly, said probes are in fluidic contact (*e.g.*, on a solid support or array, such as a micro- or nano-array, to which they are (covalently) linked)

The means of the application allow detecting that a product, more particularly a product intended for administration to a human (*e.g.*, a product intended for administration to a human *via* the oral or intra-gastric route or the intra-peritoneal route, more particularly *via* the oral or intra-gastric route, such as a food or beverage intended for human consumption) is pathogenic to human. Indeed, the means of the application allow detecting that said product contains a *L. monocytogenes* isolate, which has a high probability of having a potentiality, more particularly a high potentiality, of causing invasive infection in a human. The means of the applications also allow detecting that the line of production of said product is contaminated with an isolate of *Listeria monocytogenes* that is pathogenic to human.

More particularly, the means of the application allow detecting that said product is highly pathogenic to human, *i.e.*, that said product contains a *L. monocytogenes* isolate, which has a high probability of having a potentiality, more particularly a high potentiality, of causing invasive infection in a human, *i.e.*, a *L. monocytogenes* isolate, which has a high probability of causing a life-threatening infection or an infection where said *L. monocytogenes* isolate is present in a normally sterile body tissue.

Said potentiality of causing an invasive infection is particularly high, where the human to which the product is intended to be administered, is an immunocompromised human, a pregnant woman, a fetus, a neonate, an infant, a child, or an elderly.

In the application, the term “neonate” encompasses a human of 1-day old to less than 4-week old, the term “infant” encompasses a human of 4-week old to less than 1-year old, the term “child” encompasses a human of 1-year old to less than 12-year old, the term “elderly” encompasses a human above 60-year old, more particularly above 65-year old, more particularly above 70-year old, more particularly above 75-year old, more particularly above 80-year old.

Said isolate of *Listeria monocytogenes* may *e.g.*, be present or detected in a product intended for administration to a human, *e.g.*, *via* the oral or intra-gastric route or the intra-peritoneal route, more particularly *via* the oral or intra-gastric route. Said isolate of *Listeria monocytogenes* may *e.g.*, be present or detected in a food or beverage product intended for human consumption.

More particularly, said isolate of *Listeria monocytogenes* may be present or detected as contaminant microorganism in a food or beverage intended for human consumption.

Said isolate of *Listeria monocytogenes* may *e.g.*, be present or detected in a tissue or body fluid of a human, more particularly of an immunocompromised human, a pregnant woman, a fetus, a neonate, an infant, a child, or an elderly, or in a sample of said tissue or body fluid. Said tissue or body fluid can *e.g.*, blood, plasma, a tissue or fluid from the gastrointestinal tract, a tissue or fluid from the brain, a tissue or fluid from the spinal cord or a tissue or fluid from the placenta.

Detecting that gene(s) is(are) present in said *L. monocytogenes* isolate may *e.g.*, be performed on a sample or non-naturally occurring sample containing nucleic acid material, which has been (previously) isolated or extracted from said isolate or which has been (previously) purified from a lysis extract of said isolate.

Nucleic acid isolation or extraction as well as nucleic acid purification are well known in the art, and include any means that the person of average skill in the art may find appropriate for nucleic acid isolation, extraction or purification from *L. monocytogenes*, such as a lysis buffer for DNA extraction, which contains Sodium Dodecyl Sulfate (SDS) and optionally sodium hydroxide.

The application also relates to oligonucleotide products as such.

The application relates to each primer, primer pair, combination of primers, combination of primer pairs, more particularly to each set or multiplex of primers or of primer pairs, which is herein described. The application relates more particularly to a set of oligonucleotides, which comprises
5 at least two, three or four primer pairs of the application.

The application relates to each probe, combination or set of probes, which is herein described.

The application relates to each combination or set of oligonucleotides comprising at least one primer pair and at least one probe of the application, more particularly at least two primer pairs and at least one probe of the application, more particularly at least two primer pairs and at least
10 two probes of the application.

More particularly, the application relates to a set of oligonucleotides (for detection of genes i.-iv.), which comprises:

- said primers for specific amplification from said gene i. of SEQ ID NO: 1, more
15 particularly the primers of SEQ ID NOs: 17 and 18,

- said primers for specific amplification from said gene ii. of SEQ ID NO: 2, more particularly the primers of SEQ ID NOs: 20 and 21,

- said primers for specific amplification from said gene iii. of SEQ ID NO: 3, more particularly the primers of SEQ ID NOs: 23 and 24, and

20 - said primers for specific amplification from said gene iv. of SEQ ID NO: 4, more particularly the primers of SEQ ID NOs: 26 and 27,

and/or which comprises

- said probe for specific hybridization on gene i. of SEQ ID NO: 1,

- said probe for specific hybridization on gene ii. of SEQ ID NO: 2,

25 - said probe for specific hybridization on gene iii. of SEQ ID NO: 3, and

- said probe for specific hybridization on gene iv. of SEQ ID NO: 4.

Said set may comprise said primers and/or probes, more particularly said primers, in mixture together in said set.

Said set may further comprises at least one primer pair or probe for the specific detection of a
30 marker control gene, more particularly of said serogroup IVb marker gene.

More particularly, the application relates to a set of oligonucleotides (for detection of genes vi.-vii.), which comprises:

- said primers for specific amplification from said gene vi. of SEQ ID NO: 5, more
35 particularly the primers of SEQ ID NOs: 29 and 30, and

- said primers for specific amplification from said gene vii. of SEQ ID NO: 6, more particularly the primers of SEQ ID NOs: 32 and 33,

and/or which comprises

- said probe for specific hybridization on gene vi. of SEQ ID NO: 5, and
- said probe for specific hybridization on gene vii. of SEQ ID NO: 6,

5 Said set may comprise said primers and/or probes, more particularly said primers, in mixture together in said set.

Said set may further comprises at least one primer pair or probe for the specific detection of a marker control gene or of a combination of control marker genes, more particularly at least one primer pair or probe for the specific detection of said lineage I marker gene and at least one primer pair or probe for the specific detection of said serogroup IIb and lineage II marker gene.

10

More particularly, the application relates to a set of oligonucleotides (for detection of genes v., viii. and ix.), which comprises:

- said primers for specific amplification from said gene v. of SEQ ID NO: 9, more particularly the primers of SEQ ID NOs: 41 and 42,
- 15 - said primers for specific amplification from said gene viii. of SEQ ID NO: 10, more particularly the primers of SEQ ID NOs: 44 and 45, and
- said primers for specific amplification from said gene ix. of SEQ ID NO: 8, more particularly the primers of SEQ ID NOs: 38 and 39,

and/or which comprises

- 20 - said probe for specific hybridization on gene v. of SEQ ID NO: 9,
- said probe for specific hybridization on gene viii. of SEQ ID NO: 10, and
- said probe for specific hybridization on gene ix. of SEQ ID NO: 8,

Said set may comprise said primers and/or probes, more particularly said primers, in mixture together in said set.

25 Said set may further comprise (for the further detection of genes x. and xi.)

- said primers for specific amplification from said gene x. of SEQ ID NO: 7, more particularly the primers of SEQ ID NOs: 35 and 36, and/or
- said primers for specific amplification from said gene xi. of SEQ ID NO: 11, more particularly the primers of SEQ ID NOs: 47 and 48,

30 and/or may further comprises

- said probe for specific hybridization on gene x. of SEQ ID NO: 7, and/or
- said probe for specific hybridization on gene xi. of SEQ ID NO: 11.

Said set may further comprises at least one primer pair or probe for the specific detection of a marker control gene, more particularly of said lineage II marker gene.

35

The application also relates to products, such as a container or kit, which comprise at least one oligonucleotide product of the application.

Said container may *e.g.*, be

- a tube or a multiwell plate suitable for nucleic acid amplification (*i.e.*, suitable for receiving a reaction mixture containing oligonucleotides, *e.g.*, primers, or primers and probes, for nucleic acid amplification); or

- 5 - a solid support such as an array (*e.g.*, a macro-array, a micro-array or a nano-array), a chip or microbeads, suitable for nucleic acid hybridization or for nucleic acid sequencing (and further suitable for containing oligonucleotides, *e.g.*, probes, (covalently) linked thereto).

The oligonucleotides or primers of a set (or multiplex) of the application may be contained in mixture in said tube or multiwell plate.

- 10 Said solid support may *e.g.*, be formed from (or comprise or essentially consist of) polymer(s) and/or plastic (in particular polystyrene), or glass, or silicon.

Said at least one oligonucleotide product of the application (*e.g.*, a set of oligonucleotides or of primers of the application) may be (covalently) linked to said solid support, *e.g.*, through a molecular linker. The oligonucleotides or primers of a set (or multiplex) of the application, which
15 are linked to said solid support, may be in fluidic contact with each other.

- A kit of the application comprises at least one oligonucleotide product of the application (more particularly, at least one set or multiplex of oligonucleotides of the application, more particularly, at least one set or multiplex of primers of the application), or comprises a container of the
20 application (*e.g.*, a tube, a multiwell plate or a solid support of the application).

- More particularly, a kit of the application comprises at least two different sets or multiplex of oligonucleotides of the application, more particularly, at least two different sets or multiplex of primers of the application, or comprises a container (*e.g.*, a tube, a multiwell plate or a solid support of the application), which contains said at least two different sets (or multiplex) of
25 oligonucleotides (or of primers) of the application

More particularly, a kit of the application comprises at least two or the three of the three following sets of oligonucleotides:

- a set of oligonucleotides for detection of genes i.-iv. as above-described,
- a set of oligonucleotides for detection of genes vi.-vii. as above-described,
- 30 - a set of oligonucleotides for detection of genes v., viii. and ix. as above-described (or a set of oligonucleotides for detection of genes v., viii. and ix. and for the further detection of genes ix. and x. as above-described).

More particularly, a kit of the application comprises three sets of oligonucleotides, wherein

- a first set is the set of oligonucleotides for detection of genes i.-iv. as above-described,
- 35 - a second set is the set of oligonucleotides for detection of genes vi.-vii. as above-described,

- a third set is the set of oligonucleotides for detection of genes v., viii. and ix. as above-described (or a set of oligonucleotides for detection of genes v., viii. and ix. and for the further detection of genes ix. and x. as above-described).

The oligonucleotides of a set can be mixed together in the set.

5 Each set of oligonucleotides may be separate or distinct from another set of oligonucleotides.

A kit of the application may further comprise an instruction leaflet and/or at least one reagent

- for determining that an isolate of *Listeria monocytogenes* has a high probability of belonging to clone CC1, to clone CC2, to clone CC4, to clone CC6, to clone CC7, to clone CC3, to clone CC5, to clone CC8 or CC16, or to clone CC155 of *L. monocytogenes*, or

10 - for determining that a *Listeria monocytogenes* isolate has a high probability of having a potentiality, more particularly a high potentiality, of causing invasive infection in a human, or

- for determining that a *Listeria monocytogenes* isolate, which belongs to one of serogroups IVb, IIb, IIa and IIc, more particularly to serogroup IVb or to serogroup IIb or to one of serogroups IIa and IIc, has a high probability of having a potentiality, more particularly a high potentiality, of causing invasive infection in a human, or

15 - for determining whether a *Listeria monocytogenes* isolate has a high or low probability of having a potentiality, more particularly a high potentiality, of causing invasive infection in a human, or

- for detecting or determining that a product intended for administration to a human (*e.g.*,
20 *via* the oral or intra-gastric route or the intra-peritoneal route, more particularly *via* the oral or intra-gastric route, such as a food or beverage intended for human consumption) is pathogenic to human (due to the presence of *Listeria monocytogenes*), more particularly pathogenic to an immunocompromised human, a pregnant woman, a fetus, a neonate, an infant, a child, or an elderly, or

25 - for implementation of a method or product of the application.

Said at least one reagent may *e.g.*, be at least one reagent suitable for DNA nucleic acid amplification, more particularly for DNA nucleic acid amplification from *L. monocytogenes*.

Said kit may *e.g.*, further comprise one or several reagents selected from

- a (DNA) polymerase from a microorganism other than *L. monocytogenes*, *e.g.*, a (DNA)
30 polymerase from *Thermus aquaticus*,

- free dNTPs,

- a buffer solution suitable for nucleic acid amplification, more particularly for PCR amplification, such as a Tris-HCl buffer suitable for nucleic acid amplification, more particularly a Tris-HCl buffer suitable for nucleic acid amplification, which is at pH 8-8.5, more particularly a Tris-HCl

35 buffer suitable for nucleic acid amplification, which is at pH 8-8.5 and contains KCl and MgCl₂,

- a cell lysis buffer for DNA extraction, such as a cell lysis buffer containing SDS or SDS and NaOH, more particularly a cell lysis buffer suitable for DNA extraction from *L. monocytogenes*.

The application also relates to

- a computer program product, for storage in a memory of a processing unit or on a removable memory support for cooperation with a reader of said processing unit, characterized in that it comprises instructions for carrying out a method of the application;
- an apparatus for carrying out a method of the application, more particularly a computer device, comprising a processing unit in the memory of which is stored the computer program product of the application, and measurement values for the presence or absence of genes as defined in the application; and
- a computer readable medium comprising software code adapted to cause the apparatus or computer device to perform a method of the application.

Said computer program product or software code may thus comprise instructions for determining that an isolate of *Listeria monocytogenes* has a high probability of belonging to clone CC1, to clone CC2, to clone CC4, to clone CC6, to clone CC7, to clone CC3, to clone CC5, to clone CC8 or CC16, or to clone CC155 of *L. monocytogenes*, wherein a high probability of belonging to one of these clones is indicative that said *L. monocytogenes* isolate has a potentiality, more particularly a high potentiality, of causing invasive infection in a human, wherein the presence of one of said genes, as defined in the application, is indicative that said *L. monocytogenes* isolate has a high probability of belonging to clone CC1, clone CC2, clone CC4, clone CC6, clone CC7, clone CC3, clone CC5, clones CC8 and CC16, or clone CC155, respectively, or

instructions for determining that a *Listeria monocytogenes* isolate, which belongs to serogroup IVb or to serogroup IIb or to one of serogroups IIa and IIc, has a potentiality, more particularly a high potentiality, of causing invasive infection in a human, wherein the presence of one of said genes, as defined in the application, is indicative that said *L. monocytogenes* isolate has a potentiality, more particularly a high potentiality, of causing invasive infection in a human.

More particularly, said computer program product or software code may comprise instructions for determining that an isolate of *Listeria monocytogenes* has a high probability of belonging to clone CC1, to clone CC2, to clone CC4, to clone CC6, to clone CC7, to clone CC3, to clone CC5, to clone CC8 or CC16, or to clone CC155 of *L. monocytogenes*, wherein a high probability of belonging to one of these clones is indicative that said *L. monocytogenes* isolate has a potentiality, more particularly a high potentiality, of causing invasive infection in a human, wherein the presence of one of said genes i.-ix. as defined in the application is indicative that said *L. monocytogenes* isolate has a high probability of belonging to clone CC1, clone CC2, clone CC4, clone CC6, clone CC7, clone CC3, clone CC5, clones CC8 and CC16, or clone CC155, respectively, or

instructions for determining that a *Listeria monocytogenes* isolate, which belongs to serogroup IVb or to serogroup IIb or to one of serogroups IIa and IIc, has a potentiality, more particularly a high potentiality, of causing invasive infection in a human, wherein the presence of one of said genes i.-iv., or of said genes vi. and vii., or of said genes v., viii. and ix., as defined in the application, is indicative that said *L. monocytogenes* isolate has a potentiality, more particularly a high potentiality, of causing invasive infection in a human.

More particularly, said computer program product or software code may further comprise instructions for determining that an isolate of *Listeria monocytogenes* has a high probability of belonging to clone CC121, or to clone CC9 of *L. monocytogenes*, wherein a high probability of belonging to one of these clones is indicative that said *L. monocytogenes* isolate does not have a potentiality, more particularly a high potentiality, of causing invasive infection in a human, wherein the presence of one of said genes x. and xi. as defined in the application is indicative that said *L. monocytogenes* isolate has a high probability of belonging to clone CC121, or clone CC9, respectively, or

instructions for determining that a *Listeria monocytogenes* isolate, which belongs to serogroup IIa or to serogroup IIc, does not have a potentiality, more particularly a high potentiality, of causing invasive infection in a human, wherein the presence of one of said genes x. and xi., as defined in the application, is indicative that said *L. monocytogenes* isolate does not have a potentiality, more particularly a high potentiality, of causing invasive infection in a human.

The term "comprising", which is synonymous with "including" or "containing", is open-ended, and does not exclude additional, unrecited element(s), ingredient(s) or method step(s), whereas the term "consisting of" is a closed term, which excludes any additional element, step, or ingredient which is not explicitly recited.

The term "essentially consisting of" is a partially open term, which does not exclude additional, unrecited element(s), step(s), or ingredient(s), as long as these additional element(s), step(s) or ingredient(s) do not materially affect the basic and novel properties of the invention.

The term "comprising" (or "comprise(s)") hence includes the term "consisting of" ("consist(s) of"), as well as the term "essentially consisting of" ("essentially consist(s) of"). Accordingly, the term "comprising" (or "comprise(s)") is, in the present application, meant as more particularly encompassing the term "consisting of" ("consist(s) of"), and the term "essentially consisting of" ("essentially consist(s) of").

In an attempt to help the reader of the present application, the description has been separated in various paragraphs or sections. These separations should not be considered as disconnecting the substance of a paragraph or section from the substance of another paragraph or section. To the contrary, the present description encompasses all the combinations of the various sections, paragraphs and sentences that can be contemplated.

Each of the relevant disclosures of all references cited herein is specifically incorporated by reference. The following examples are offered by way of illustration, and not by way of limitation.

EXAMPLES

5

Example 1: Identification of hyper- and hypo-virulent clones of *L. monocytogenes*

The French National Reference Center of Listeria (NRCL) receives 99% of the isolates involved in clinical infections in France as well as 80% of the food isolates from official controls and own-
10 checks by Industry that do not comply with European regulations. Consequently, the NRCL's strain collection contains a pool of strains representative of the *L. monocytogenes* isolates in contact with humans and of the isolates that really infect patients.

The MLST clone for over 6,000 surveillance isolates isolated between 2005 and 2013 from human infection cases (40%) and from food sources (60%) was determined. Among the isolates that were
15 analyzed, 81% were represented only by 13 MLST clones that we considered as major clones (Figure 3). Some of them were significantly associated to clinical or food origins.

As expected, at lineage level, the proportion of food and clinical isolates were significantly different between both lineages, with 56% of the lineage I isolates but only 24% of the lineage II isolates being of clinical origin ($p < 1.10^{-155}$). Similarly, significant differences were also observed
20 between IVb and IIb serogroups, as 62% and 42% of the isolates were of clinical origin, respectively ($p < 1.10^{-22}$). These results confirm the common view that lineage I and serotype 4b are associated with infections.

As compared to the entire dataset, lineage I clones CC1, CC2, CC4, and CC6 ($p < 1.10^{-4}$), as well as the lineage II clones CC101-90, CC207 (P-values $< 1.10^{-3}$) and CC7 ($p < 1.10^{-4}$) were
25 significantly associated to a clinical origin. In contrast, the lineage II clones CC121, CC9, CC31 (P-values $< 1.10^{-4}$) and CC204 ($p < 1.10^{-3}$) were significantly associated to a food origin. In addition, CC1, CC2, CC4 and CC6 represented almost half of the clinical isolates, and CC9 and CC121 more than 40% of the food isolates (Figures 4A, 4B and 4C).

At the lineage level, the clones CC1 and CC4 (lineage I) as well as CC101-90, CC155, CC207, CC7, CC8-16 and ST504 (lineage II) were significantly associated to clinical origin ($p < 1.10^{-4}$). In
30 contrast, the clones CC5 and CC3 (lineage I) as well as CC121, CC31 and CC9 (lineage II) were significantly associated to a food origin ($p < 1.10^{-4}$), as compared to other clones of their respective lineage.

Eleven of the thirteen major clones are the most frequently isolates from clinical infections, *i.e.*,
35 clones CC1, CC2, CC4, CC6, CC3, CC5, CC121, CC155, CC7, CC8-16 and CC9 (Figure 1, top panel). Clones CC8 and CC16 have highly similar Pulsed Field Electrophoresis (PFGE) profiles;

they were therefore merged for clone identification and were considered as a single clone (CC8-16).

Table 1 below shows the correspondence of the (eleven) CC clones with the lineage and serotype classifications as well as with the serogrouping profile (as established by the reference method of multiplex PCR serogrouping of Doumith *et al.* 2004).

Table 1:

Clones [&]	Main serotypes [§]	Serogroup [‡]	Lineage [*]
CC1 ; CC2 ; CC4 ; CC6	4b ; 4d ; 4e	IVb	I
CC3 ; CC5	1/2b ; 3b	IIb	
CC7 ; CC8 and CC16 ; CC121 ; CC155	1/2a ; 3a	IIa	II
CC9	1/2c ; 3c	IIc	

[*] as determined by sequence-based studies (*cf. e.g., Ward et al.* 2008, Ragon *et al.*, 2008).

[§] as determined by serotyping of somatic and flagellar antigens (*cf. e.g., Seeliger and Hohne* 1979).

[‡] as determined by multiplex PCR serogrouping of Doumith *et al.* 2004.

[&] defined as MLST clonal complexes as described in Ragon *et al.* 2008.

Clones CC1, CC2, CC4, CC6, and CC7, and the group consisting of the other strains from serogroup IVb “group “other IVb”) were significantly associated to clinical origin ($p < 1.10^{-4}$). These clones and the group “other IVb” have a high potentiality of causing an invasive infection in a human (and are therefore classified as hyperinfective) (Figure 2A and 2B).

Clones CC155, CC8-16, CC3, and CC5, and the group consisting of the other strains from serogroup IIb “group “other IIb”) were not significantly associated to either clinical or food origin. These clones and the group “other IIb” still have a potentiality of causing an invasive infection in a human (Figure 2A and 2B).

Clones CC9 and CC121, and the group consisting of the other strains from lineage II (“group “other IIa”) were significantly associated to food origin ($p < 1.10^{-5}$). These clones and the group “other IIa” have a low potentiality of causing an invasive infection in a human (and are therefore classified as hypoinfective) (Figure 2A and 2B).

Example 2: Clone-specific target genes to identify major clones of *Listeria monocytogenes*

Clone-specific target genes to identify major clones of *L. monocytogenes* were identified based on a comparative genomics analysis. The objective was to identify genes that are specifically present in the clones of interest, and totally absent from all the other clones.

5 For this purpose, it was important to use a set of genomes that covers the diversity of *L. monocytogenes* clones. As no such set of genome existed in public databases, we constructed our own genome dataset. We therefore selected and sequenced by ILLUMINA® a set of 69 strains that represented the species diversity based on their MultiLocus Sequence Typing (MLST) and Pulsed Field Gel Electrophoresis (PFGE) types. A minimum of two strains with distinct frequent
10 PFGE profiles (considering the two enzymes *AscI* and *ApaI*) and isolated from distinct sources have been selected per major clone, when it was possible. They comprised 37 strains that were isolated from human clinical infections, 16 from animal infections, 12 from food sources, 1 from the environment and 3 from unknown origin (*i.e.*, 69 isolated strains). We also included in the analysis 35 genomes from public databases that were of good quality, but did not represent all
15 important clones of *L. monocytogenes*.

The global view of the diversity covered by the 104 sequenced strains is illustrated in the attached minimum spanning tree (Figure 5). The 104 genomes covered the breadth of diversity of *L. monocytogenes* and comprised 41 genomes from lineage I, 57 from lineage II, 5 from lineage III and 1 from lineage IV strains. Clones CC8 and CC16 were considered as a unique clone (CC8-16)
20 since strains belonging to these clones have such similar PFGE profiles that it is difficult to discriminate them only on the PFGE profile criteria. Moreover, no genome was available for CC16 at the time of the analysis.

Genes that are specific for eleven major clones CC1, CC2, CC4, CC6, CC3, CC5, CC7, CC8, CC121, CC155 and CC9, lineages or serogroups were identified, representing putative target
25 genes for PCR identification. Those clone-specific genes that are not present on mobile genetic elements were selected: *cf.* Table 2 below. Some of these genes allowed the production of primers that are suitable for multiplex PCR (*cf.* example 3 below). These genes are listed in Table 4 below. Control genes, *i.e.*, genes specific for lineage I, serogroup IVb, serogroup IIb and lineage II, were selected: *cf.* Table 3 below. Table 5 below shows control genes that allowed the production of
30 primers that are suitable for multiplex PCR (*cf.* example 3 below).

Table 2:

CLONES	GENES	
	(clone-specific genes that are not present on mobile elements)	
	NAME	SEQ ID NO:
CC1	<i>LMOJ2365_2702</i>	1

	<i>LMOJ2365_0687</i>	61
	<i>LMOJ2365_2701</i>	62
	<i>LMOJ2365_2703</i>	63
	<i>LISMF_2745</i>	64
	<i>LMOJ2365_2704</i>	65
	<i>LMOJ2365_2705</i>	66
	<i>LMOJ2365_2706</i>	67
	<i>LISMF_2749</i>	68
	<i>LISMF_2841</i>	69
	<i>LISMF_2842</i>	70
	<i>LMOJ2365_2799</i>	71
CC2	<i>LMOATCC19117_2417</i>	2
	<i>LMOATCC19117_0336</i>	72
	<i>LMOATCC19117_0355</i>	73
	<i>LMOATCC19117_0356</i>	74
	<i>LMOATCC19117_1127</i>	75
	<i>LMOATCC19117_1128</i>	76
	<i>LMA19117_1703</i>	77
	<i>LMOATCC19117_2382</i>	78
	<i>LMOATCC19117_2383</i>	79
CC4	<i>LMOL312_1102</i>	3
	<i>Lm4b_00679</i>	80
	<i>Lm4b_00680</i>	81
	<i>Lm4b_00681</i>	82
	<i>Lm4b_01121</i>	83
	<i>LMOL312_0396</i>	84
	<i>LMOL312_0397</i>	85
CC6	<i>LM1816_RS00050</i>	4
	<i>0600581v1_10346</i>	86
	<i>0600581v1_10347</i>	87
	<i>0600581v1_10348</i>	88
	<i>0600581v1_10349</i>	89
	<i>0600581v1_10350</i>	90
	<i>0600581v1_10351</i>	91
	<i>0600581v1_10352</i>	92

	<i>0600581v1_10353</i>	93
	<i>0600581v1_10355</i>	94
	<i>0600581v1_10356</i>	95
	<i>0600581v1_10357</i>	96
	<i>0600581v1_10358</i>	97
	<i>0600581v1_70288</i>	98
	<i>0600581v1_140005</i>	99
	<i>0600581v1_140006</i>	100
	<i>0600581v1_140042</i>	101
	<i>0600581v1_140049</i>	102
	<i>0600581v1_210015</i>	103
	<i>0600581v1_210016</i>	104
	<i>0600581v1_240129</i>	105
	<i>0600581v1_280080</i>	106
CC3	<i>LMOSLCC2482_0307</i>	5
	<i>SLCC2482_0277</i>	107
	<i>LMOSLCC2482_0304</i>	108
	<i>LMOSLCC2482_0305</i>	109
	<i>LMOSLCC2482_0306</i>	110
	<i>LMOSLCC2482_1467</i>	111
CC5	<i>M637_08170</i>	6
	<i>0500704v1_110453</i>	112
	<i>0500704v1_110642</i>	113
	<i>0500704v1_110643</i>	114
	<i>0500704v1_110644</i>	115
	<i>0500704v1_110646</i>	116
	<i>0500704v1_110647</i>	117
	<i>0500704v1_110648</i>	118
	<i>0500704v1_111156</i>	119
	<i>0500704v1_111157</i>	120
	<i>0500704v1_160076</i>	121
	<i>0500704v1_180010</i>	122
CC121	<i>LM6179_0610</i>	7
	<i>070104v1_70003</i>	123
	<i>070104v1_110043</i>	124

	<i>070104v1_110044</i>	125
	<i>070104v1_190011</i>	126
	<i>070104v1_210032</i>	127
	<i>070104v1_490004</i>	128
CC155	<i>LMLG_RS02400</i>	8
	<i>LISMN_0064</i>	129
	<i>LMLG_2609</i>	130
	<i>LISMN_0816</i>	131
	<i>LMLG_1649</i>	132
	<i>LMLG_1650</i>	133
	<i>LMLG_2951</i>	134
	<i>LMLG_1653</i>	135
	<i>LISMN_0842</i>	136
	<i>LISMN_2393</i>	137
	<i>LISMN_2481</i>	138
	<i>LMLG_3016</i>	139
	<i>LISMN_2757</i>	140
	<i>LMLG_0301</i>	141
CC7	<i>LMRG_02577</i>	9
	<i>LM03S_0078</i>	142
	<i>LMRG_02892</i>	143
	<i>LMRG_02891</i>	144
	<i>LMRG_02937</i>	145
	<i>LM03S_0305</i>	146
	<i>LMRG_02934</i>	147
	<i>LMRG_02576</i>	148
	<i>LMRG_02575</i>	149
CC8 and/or (and) CC16	<i>LM5578_1185</i>	10
	<i>LISM1_0269</i>	150
	<i>LISM1_1210</i>	151
	<i>LM5578_1182</i>	152
	<i>LM5578_1183</i>	153
	<i>LISM1_1214</i>	154
	<i>LISM1_1215</i>	155
CC9	<i>lmo1118</i>	11

Table 3:

CONTROL GENES (genes with lineage/subgroup specificity)		
SPECIFICITY	NAME	SEQ ID NO:
Lineage I	<i>LMOJ2365_2638</i>	12
	<i>LMOJ2365_2058</i>	156
	<i>LISMF_2812</i>	157
Lineage II	<i>lmo0525</i>	13
	<i>LISIN_0086</i>	158
	<i>LISIN_0633</i>	159
	<i>lmo0734</i>	160
	<i>lmo0735</i>	161
	<i>lmo0737</i>	162
	<i>lmo1060</i>	163
	<i>lmo1061</i>	164
	<i>lmo1062</i>	165
	<i>LISIN_1421</i>	166
	<i>lmo1968</i>	167
	<i>lmo1971</i>	168
	<i>lmo1973</i>	169
	<i>lmo1974</i>	170
	<i>LISIN_2953</i>	171
Serogroup IIb and lineage II	<i>lmo1077</i>	14
	<i>lmo1076</i>	172
	<i>lmo1079</i>	173
	<i>lmo1080</i>	174
	<i>lmo1081</i>	175
	<i>lmo1082</i>	176
	<i>lmo1083</i>	177
	<i>lmo1084</i>	178
	<i>lmo1085</i>	179
Serogroup IVb	<i>LMOJ2365_1900</i>	15
	<i>LMOJ2365_2740</i>	180
	<i>LMOJ2365_2741</i>	181

Table 4:

CLONES	SELECTED GENES (clone-specific genes that are not present on mobile elements and that allowed the production of primers for multiplex PCR)		
	NAME	GENBANK® accession number	SEQ ID NO:
CC1	<i>LMOF2365_2702</i>	AE017262.2 ; complement(2749802..2751088)	1
CC2	<i>LMOATCC19117_2417</i>	NC_018584.1 ; complement(2492236..2495424)	2
CC4	<i>LMOL312_1102</i>	NC_018642.1 ; complement(1135287..1136126)	3
CC6	<i>LM1816_RS00050</i>	NC_021829.1 ; complement(7752..8792)	4
CC3	<i>LMOSLCC2482_0307</i>	NC_018591.1 ; (334781..336511)	5
CC5	<i>M637_08170</i>	CP006592.1 ; (1638443..1639534)	6
CC121	<i>LM6179_0610</i>	NZ_HG813249.1 ; complement(614809..616449)	7
CC155	<i>LMLG_RS02400</i>	NC_017547.1 ; (496745..497509)	8
CC7	<i>LMRG_02577</i>	NC_017544.1 ; complement(317879..318991)	9
CC8 and/or (and) CC16	<i>LM5578_1185</i>	NC_013766.2 ; complement(1175757..1177562)	10
CC9	<i>lmo1118</i>	NC_003210.1 ; complement(1154864..1156381)	11

Table 5:

SELECTED CONTROL GENES (genes with lineage/subgroup specificity that allowed the production of primers for multiplex PCR)			
SPECIFICITY	NAME	GENBANK® accession number	SEQ ID NO:
Lineage I	<i>LMOF2365_2638</i>	AE017262.2 ; (2684358..2685827)	12
Lineage II	<i>lmo0525</i>	NC_003210.1 ;	13

		(560443..561774)	
Serogroup IIb and lineage II	<i>lmo1077</i>	NC_003210.1 ; (1107975..1109663)	14
Serogroup IVb	<i>LMOj2365_1900</i>	AE017262.2 ; complement(1924279..1925631)	15

Sequences SEQ ID NOs: 1-15 are as follows (in the 5'-3' orientation):

SEQ ID NO: 1

5 ATGGGGAAATTGTTTATTAATGCAACAAAAAATTTTGGCAATAAAATAGAAATAGCA
ATCAGCAACTCAAACGGAAGAATCGATGGAGTACACTATAGTATAGCTGATATACCA
ATAGAAAAAACTTTTGAATTGAGAAGTGAGGGTATTTACACTTTTCCTGAACTACCTC
TTTACTATCTAACCACAAAAAATGATTATATTGAAAAATATTTGGATCGATCACCAAT
TAATTTAGAGGATGATAAAAGCTTTATCCCTAACGAAAGAAATAACAAGCTTATCTGC
10 ATGGAAAAGCATATTGATTTATCATTTTTAAGTACGCTACCATTATCAACTTTTGAGTA
TTCTACAAAACGTGAAGAATTTTTAAATATGGTTAATACATTTTATGATACTTATTACG
AAGATGATTATCTTATGACGCCGACATTTATGATTAATAAAGTCGGTTCGTGGGAAGA
GATTATGTTGCTAGATGTTTTAAAAAATACAAATGAATATCTAATTTCTAATCAGAAA
AGTAACGTTTTTTTGAGTATATTACTTGATCCAGTGCTTTTGGATTCTTCGGATATTGC
15 AGGTAATATCTTGAACATATATCGACATGTATCCTTTGATAAACAACCTTCAGTTTAACT
ATAATTAGTGATGAGTCGATGTATTATCATAACAGAAGATAATTATAAAAATATACTTA
TTTTTATAAAGGAGTTAAGAGCTCGAAATATAGAAGTCTACTTGCAATATTGTGGAAT
TAAAGATGTTGTTTTTTTCAGTGTTGAATATAAAAAGATTTTCAGTAGGTTGGTTTGGA
AGTTATAGAAATTTTGATACTAATTCAAAGAGAATTTTCAGAAGTGAGCTCCAGTTCCT
20 TTGGAAAGAGAATCAGAAAAATATTGACAGAAAAATTTTATGTCAGAGGTGCCGTTAA
GCTATTTAAGTGTTTTAAATGAAAGTGAATGTAAGAAATATTTTCTATTGAAAAAGA
TAAACTTGAACTATTGACTATAAGGACCTGGAACAAAGATACTGGTGTGAGATGTT
AAAAATTATAGAAGAAGACACTTCTGACGAGAATCTTTATACTGGAGATGAGTTAAT
AACTATAGATGTGAAAGAATTAATAAATAAAATTTTACAATGCAGTTTCCAATTTGTCT
25 TTTTGTGATAAGAAAACATAAAGAAGCAGGACGATTTGCGGATGCTACGAAATTAGAA
AAAAATAATTTGAAACATGTAAACTTTATGAAGAAGTTTTAAATGATTTTCAGGAAA
AATTATTCTTTTAA

SEQ ID NO: 2

30 ATGGTTCAATATTATACAGATAAAGAATTTACAGACTTGACACATACACTTGCAAAGG
CAGTAAAGGATTTTAATCTAGTTGTTTTCGTAGGAGCAGGAACCTCTTTATCACAAGG

ATATCCAAATTGGAATGGTTATATTGAGAAGCTAATTCATTTCTGGCAGTTCAATATT
CAACATGTTGCTGGCGGGAAAGTTACAGTGACAAATGAGTTGCTTTCGCAATTTGATG
AAATCTTGAATTCAAATACTACACCAAAAAGAAAAATTGATTTATTGCACACTTTGTT
ACAGGATATTTTGAAGAAGACTTTTAAAGAGGTAAAGCTTAATTTTGAAGAGTATTTT
5 TTCAAAGATGTTGTACCAGATTACATAGAAAATAGTGTGTTAGTTGAAATGATAAAAT
TAGATCCAATCTTTATCACTTCGAATTATGATTTTGAAATTGAAAGACATTTGAAGGTT
TCAAAACAAAAAGGAGCATTCAAACCAATAAATAATATTCGTGAGTTTGTAGAATCA
AATAATAAGTTACGTTACAGGCGATGTTTTACATTTACATGGAACCACTCAGGGAAATT
GGGATTTTTTTTGTCAATTCATCAGTTGATTATTCTCGACAGTATTTAAAAGAGTCAAGC
10 GACTTCAATAGTTTAAAGTAAATGGTTTCAAGAAAGAAATCCAGTTGTTCTATTTATTG
GAAGTAGTATGGAAGAAGAGGAAATTTTATCTCTACTTCCAGCCACTACTAAAAATTT
CGCACTGATGAAAGCAAATTCAAATGAGACACGTAGTTTTAGAAAAATCTACAATAA
GACATATCAAAATAACAATAATACAACTATTTTTTTGGTACGGAGATAGCTATGATGAT
TTACCTGGAAAAGTATCTGAAATTGTAAAGGCTATCGAAAATGAATTAGAAATTCAC
15 AAAGCATTGATGATTGGAATATACTCCATTCAGTATCTACTAAGGATAAAATATATAA
GGAAATTCCTGAAAAGCATATAGAAGATAAGAGATTTTTATTTGATATTTTTTAAAGCA
GAAGATCCTAATTTAGAAAAAAAGATTTTAAAGAATACATTTAGCAGTCATATTTTAT
TAGATAAAATTAGTGATATTAGTAGTTTTTGGACAATGATTGATAAAAAATTTTGAAAC
TTTAGATGAAGAACAAGTTCGATCGATTATTAGCATTTTCCAAAAGCAGAGCTTGAGC
20 GTTTATTGGAAGGAAATCTTTAAAGTTTTTGGAGAAATTAAAAGAATCAGAACCTATCG
GACAAGATGATATAAATAAAATGAGAAGAAATCTTTCCCAAGAGCAAGAAATTATTG
AAACAGCTTTCTCTAGTGATGCTGATTTAATGGGTTATTGGTTAATTGAACAACCTTCA
AAAGGAAACTTCAAATCGTAGTAGTATTTTTTATGATGATGAGATACTTTCAATTAAT
TTGAAATCTGAAATGATTCCATTAATTGTTAAACTAATGACGGATGAACTAGATATA
25 TTTATTGGTCTTTTAAAGAAATTATCTCTGATGAGCTGATAAGAATTATATATGTATCT
CTTCTGAATGACAAAATGTTGTTGGATAATAAGTCAATATTAGATAATTGTCCTGACT
TATTACTAGAATCTCATCCTTTCCAAAGAATTTTAGTAAGTATTGATAATGAGGTTGG
ATTGAATGATTCTATTAAAAATAAGTTGATAACTAAAATTGATTTTTCCAATACTACCT
TTGGAAGCGAGCTAAACCTTTTTTCAAGAAAACACAAAGATGAGATTGAAGAATTAG
30 GGATAGAAATTTCAAGAGATTATCAAGATATGATTTTTTGGAGTTGAGTCTGGTTTTGT
ACATCAGAAATCATTTATTGATATTAATCAGATTCTTAGTGAAGATATGGATGCTATT
TTGGAAATTTTTATTACCCAAACAAGATGAGCTTAGTTCAAAAAGAGAAGACTTTTTTT
CTGAAAGCACATATCAAGAAACATCTAATTTTTTTATTAAGCTTGTTGAATAAAAATGA
TGAAGTATCAAAAAAAGTAAAACAGATAATTCTTGAAAAAGGATTACTTTTGTATCCA
35 ATATATGACAAACTTTTCGTTGAGATTTTAGTAAATAATACCTATTGTACTGAATTAA
GAAATGAATCTCTGAATATCTTCTTAGAGAAGTTCAGTTTGAAGAGTTTTAGTTGGGA
AGAGAAGAAATTTTTCGAAGCATTAAATTGATAAAGAAGAATTTGAGAACCTAGCTTTT

GAAAAGCTACTAAAAGTAAATGTTAATGAGTTGAACTATGATTATGTTTTTGTAGATA
AAACAAGACCAGAATTACTAGAAGTCAATGATTTTATTAACACGGAAGTACTAGGAAGAT
ATTTAGAGATACTAATAAAATTGAATAAAAAAGAGTACTCTAGGCGGAGTGAAATTA
AAAATATTATATCTCAAGTAAATTCAAACCATTTAAAGAATTTTCACAAGGTGCTTT
5 ATCATTAGTGAACAGTTCTGTAGACTTAGAAGAAATTACTATTAATACCTTCCAAGGC
TATTCTTATGTTGTTCTGTTGATTTCAAGAAAAAAGTTTGGAAAAATTTAAATCTGTAG
GTCAAGAGTTATTAAAAAAAGGGCTTGTAATGACTTGAATAAAGATAATCTATTTAT
TCTCTCTCTATCCATGATAAATCCGAGGGAGGAAGAAATGGAGATTAATTGGAGTGA
AATTAATTTTTCAAGGATTTTTGATATCATTCTTCAAAATGAACTTGAGTTCGCTTTTG
10 AGTCACAATGGATTGAAAATATAATTTTAAAGGATGAAGATGGACAATATGGGGTGA
GTATCTTATATTCTATCAAAGAGAGCAAGCTTTAATAGATAAGTCAAAAAAGCTTGT
TAATATATTCGAAAAAGAAATTAGTAATTATAGTGGGGAAATAAAAGTTGATTTGTTA
CCGCATGCTATTAAAAAACAAGACAATCCACAAAAAAGGATTTATTGATTAGATTCT
TCTTTATATTACTAGATAATGCTAACTTGCTCAGAGTTATTTTGGAAAGTGGGATGTTA
15 GCAGATTTAATGATGCACTTGAATTCTAGTTTACAGAAAAAAGTTGCCAAACATCCTA
AGTTATCGACTATTCTATCACCATTAGAAATTGAAAATTTGAAAAGAGAGATTGAATA
A

SEQ ID NO: 3

20 ATGAATCAATTACAATACTCTGAAGATGCTTTAAAAAATAGGTATCTCTTTTTTAAAG
AAATAAATGAAATAAATATTTTTGTTGAAGATAAAGGAAAAGAATATGAATATGAGG
AAATATTGTCTAAAGTTTTTGCTGATGAATATCATATACAGACGATATATGCACTGGG
GGGTAAACCACAAGTTTTATCCGCATTTAGAGACTGTAAAGAACATGTTTCTAGCAAT
AATAATATAAAAAACATATATCTTTTAGATGGTGATTTTGATCAATATTTGGATAATA
25 TAGTAATGGAATCACATCCACATATTATTTATTTGCAATCATATAATATTGAAAATTAT
TTTTTGAATGAAGAATCGGTAGTAACTTTTATGCAAGGAAAAATGAAAATGCTGAAG
AATGATGTAAGGACTAAAATAGCTTATTCTGAATGGCTTTCTAATACATTATACAATA
TAGAAAACATTTTTATTTTATATTGTATAATTCAAGAAAAGGAATTAGGGATTCCTAA
TGTAGGGGATTCTGAATATAAAGTAATTAATATGATCGATGGATCTATCAATATGACG
30 AGATATGAGGATTTGAAAAAGCAAATAAGTAATTGTACAGTAAACATAGATTTAGTA
GAGCAAGAAATGAAAGCAAAAATTCACAATATAAATCCTAATGTGTTTGATTTGATTT
GTGGTAAACATTTGATTTGTAGTTTGTACCGTCATCTGTTAAAAATAACTAAAGGTTTC
AGCTATGATGAATTTAGATGGCATTTAGTTCAAAATGTTGAAGTATCTAGACTTAATT
TTATAAAGGAAAGAGTTGCTAATTTGTAA

35

SEQ ID NO: 4

ATGAAGAAAAAGAATGTATTAAATTTAATTAAATATCATTTCAGAAAAAATGATTTTC
AGTTTAGAAATGAGGCTATCGAAATTGCTCGCTATTTTGATACAATAGGTGACAGTCA
ATTATCGGAATACATCATGAGTTTGTATCTACAGCAAATACCTTTGTGCCGCAAGGT
GAGGCTTTTGAGAACTTATTTTTCAAAAAAATAGAACCTAATGTAGAACCATTACCAC
5 TCCCTAAAGCAATTAGTGAAGATATTCGAGGTATTATAAATGCTGTAAATCATCATGT
AGGAATAAATAAATTTCTCTTCGAGGGTGCACCTGGAAGTGGAAAGACAGAAACGGT
TAAACAGATTGCTCGATTATTAGAACGCCAACTATATATGGTAGAATTTAGCGAATTA
ATTGATAGTAAATGGGGCAAACAGCTAAGAACATTGTAGCAGTGTTCAACGAAATA
AATCGTGTATCAAATCCATCAAAATTATTAATACTTTTCGATGAAATAGATGCTATAG
10 CCCTAGATAGAGTAACTCTAATGACTTAAGAGAAATGGGACGGGTAAACATCTACAA
TCTTGAAAGAGTTGGATAAGTTAAATGAAGAAGTTGTTCTTATTGCCACTACTAATTT
GTATGAAAAATTTGATAGAGCCTTAATAAGAAGATTTGATTCAGTAATTAATTTAAT
AGATATGAAAAGGAAGATTTAGTAGAAATTTTCAGAAATCATATTGAATTCATTATTGA
AAAAATTTAAAGATGCTGGACGAGATATGAAGTTATTTAAAAAGTAATCAGAAACA
15 TGGATGAAATTCCGAATCCTGGAGAATTAAAAAATTTGATTAGGGTATCATTAGCTTT
TAGTGATCCAACAAATGAATTTGATTATCTCAGGAGATTGCTGAGGTATGTAGTTGAA
AAACCTGGTAATATAGAATTGAAAGAGTTACAAAACATGGGGTTTACTGTTAGAGAG
ATAGAGATATTAAGTGGTATATCAAAAAGCCAAGTCTCAAGAGAGTTGAAGGGAGAT
TGA

20

SEQ ID NO: 5

ATGAGGAGAAATATAAGTAGCATTGATTATGATATTCTGGAGGAGTGTGCTCTAAAAT
ATTTTGAAAAAGTAGAAAAAACAACAAATCATTAGATAACAAAAATAAAGCTAGA
ATAGGATTTTATTACTTAGTTTTTGATTTACTCTATAATATGAATCAGGTGGAAGAAGT
25 GAATACGTGCATTACAGATCAGACTTTCAGTAATATAATGAGACAACAAGATAATAA
AGATTTAGGCATTGATGCTGTATACATAGATGAAGAGAATAAGGAAGTTAATTTTTTT
AATTTTAAATATCGAGAAAAATTTATAAAAGGTAAGACTAAAGCCCATGATGAATTA
AGAGCGTGTGAACCATTTTTACTTTATGTTTCAGGATACAAATAGTTTTGAAAAAGATA
AGTCAGAATTAAGTAATTTTACTAGAGAAAAATTAAATGATATAGCTGATGTGAAAA
30 AAGATTCACAATACTCATATAAGCTATATATGGTAACAAATGATATGACTACTACAGA
TATTGAGGAGCCACAAATAACAGCGTTTAAAAAGAACTATAGTTGGTTAGAAGTTGA
GGATATCAATTTAGCTACTATAAGCGAATGGGTATCTATTCGTTCAAAGGAAAATGTT
TCTATATTAGTATTAGAGAACGAGTCTCTTTTACAACATGGTACGTCATACGAGACAG
CTAATTCATATGTTGCAGAGGTTTCTTTAGTTGAATTAATAAAGATGACAAGTCAAAA
35 TATCGATTTGCGAATGAAAGAAAATATTGATGATCTTAGCATTGAAGAAGTTGCTGAT
TTAGAAATTGATTTAGATGTTTTGTTTGATAATGTCAGAGGGTATCTAGGAAACACAA
ATTATAATAAAAAAATTCTTGAAACTTTGAAAAAAGAACCTGGAAAATTCTTTTTATT

TAACAATGGTATAACAATTACTGCAGATGATATTAATGTGAAAGAAGTTAGACTGGG
TAAGGCTAGTCGAATTAAGCTAGATCGGTTTCAAGTTGTCAATGGGGGACAATCCTTA
CGAACTATATATAAATACAAAGATCAAAAAGACTCACCAATTGAAAATTTAGCTAAG
GCAAGTGTATTAGTGAGATTTTTTAAAACAGGGCAAGAAGAGGGATTAATTAATAAG
5 GTATCAGAATATACCAATAGTCAGAATGCTATCTCAGCTATTGATTTAAGGACAGTTG
ATCAATTACAATTAATAAATTGAGAAGCACTTAGAACTCAAGGGAATTCAATATATAA
GAAAGAGAAGTTCTGATAAACTAAGTTGCAACTAGAAAATTCTATTACAATGGAAA
AACTAGGCCAATTGTTATTTGCATACGCGGGTTATCCTGAAAAAGCAGGGAATAATA
AGAGGAAAATATTTAGTGAATATTACGAGAAAGTATTTACTAATGAAAGTGATTTGAT
10 GGATAGAATTATTTCTACTATAGATACTTATCATAAAATAATTTCTTTATATAATGATG
ATGACTACAAATATTATGAGCAGAAAGTTTATTATATAATTTATTTAAAAGATTTTT
ATCAAATAAAAATTTGAAAGAGTGCTTAGAGCTTTTTGAGGATGTTTTGCTCAATTAT
GAAACCAAACAAGAAGTATCCCCCTCTAGAAAGCTATTACAATCTAAATTTAAAGAG
GAAATAAATAGAATAATTAAGTTGGATATAGGAGATATAAAGGGTCTAATTAATTTG
15 TAG

SEQ ID NO: 6

GTGAAAAAAGTTGGGAGAAATGAATTATGTCCATGTGGTAGCGGGAAGAAATTTAAA
AGATGCCACCTTGGTGTTAATGAAATAGATGAAGATAATATGGAAGACTTTGTTAAG
20 ACTTATAAACAATTAAATAAAGAAAGGCGAATAAAACAATGCTTATATCCAGAAAAA
TCTAAATGCAGTGATAAAATAATTAGAGCGCATTCTATACAAAATAGTAAATAATTC
AACGAATATCTGAGAATGGGAATGTTTATATGAACAAAGCAAAACCAAATAATCCAT
TTGCTGTATTTACTAAGTGGGGGCGTAAAGAAGCAACGACATTTACTGGATTTTGTGA
TTATCATGATAAAGTTGTATTCCAGCCAATTGAAGATAGAGACTTTGAGTTAAGTAAT
25 GAACAGATCTTTTTGCATACTTATAGATGTTTGGTAGCTAACATGCATGATAAGCAAG
AACAAGTTAAGTTAGGGAAATCAACTACTGAACTTTTTTCATTATCCAGTGAATCTAG
TGTTGGATTATTGGGCTTAGGAAATGGCTTGGCAGTGGATGATTTCTTGCGAGAAAAA
ATGATTTTGGATAAGGCTTTAATAAATGGGAGACTTTGATGTAGTTGATTCAATTGTAT
GGGAATTTGATTTTCCCATACAGTTTGCAGGTAGCGCATTGAAGCTCCTACACATGA
30 CTTGAAAAATAGAAAGATACAATCTCTCGAAACCAATGATATTATAGCTCATTTATTT
ATTACTATTTTTCCAGAAAATGATAAATCATATTGTATCATTTTCATGGCTAAAAAATTC
GAAAGAGATTCTTAGTGAGTTAGTTGAACAAATTATGGGGCTTAGTGCGAAGGAAAG
AAAAGATTATTTAAACACAACCTTTAGTAAAAAATTCGGATAATCTTGTTTTTAGTCCT
AAATTGATTGAAAGAATGTCAAAAGATGAAGAGCATTACTTTGGGTATACTTCTCAGG
35 GAGCCTATCTATTCGAGGCATTACTTGAAGCGATGGATGATGAACTTCATATTGAATC
TAAGATCCCAGAGGTAAGTGATCCAGGTTATGATTTATTCAAGCAATGTAA

SEQ ID NO: 7

ATGGTCTCAAGTAAATTAAGAACTACCTGGTTTGTAACCTCGTCCTCAACGTGATCCTC
AATTTTTTTCACAAGCATTAGAAGCTTTAAAAAAGCAACGAGTAATTTAATGAAAA
ATGGTCCTCTAATAGAGAAATCCATAAAAAATATGAGAAATTTCTAGCAGAGAATGG
5 GCTAAAAAAGAAAAATATTTCTTCTGATGGATCCGGCGGTCTGAACTTGGGTGCTATG
TTAAGAACATATAGCCTGGTTTATATAGATCCGAACTCTGGTAACTCATCCCCACTA
AAGTAGGTATAGCTTTACTTAAACGGGAAAAAAGATATGAAAATGTGACGAAACAAC
TACTTACTTTACAAATCCCAAATGCATATTTTGTTCCTTCAGGCTTTAGACCTAAGTAT
GATAAAGGCTTCTCAATTCGTCCTATTCGCTTTTTAATAAAGTTAACATGTCAAAGTTC
10 TTTAGAGTATTATATCACTAAAGAAGAAATTATTTTTTTTGCTATGACCGCAAAACAA
GATTGTGAGCTTGATGCAGTAATAGAAAGAATTAAAGATTTTAGGCTTAGTGATTCTGA
AAAAAAGAAATTATTTAAACAAAGTATAGCTACAATTGATTATCGGGAACGATCAG
ATAAAGGAGCCAGAGGTTTTGAAGAAGCTAATGGAGATGTAGCTCACACTCTAATGT
TACAAGCTAGTTATACAGAATTGGTGAAATACTCTGCTGGAAAATTAATAATAGAAC
15 AGAGCCCTATTGAATTTAAAAAAGTGATGCAACACTATCAGAATATGATGAGAGGT
ACCCATTTCGATAGTCGTTATGAAATTTCTGAACCCAGATTTAGTGAACATGCTGGTTT
AGACATTGATAGTTATAAATCTAGGCCTATTGTAAATACTAAAGTTGCCAGCAATCAG
CAAAAAATAGACCTACAGTTAAAAAATCGTGTCTAAATCACCTAACTTAATTGGCC
AAGATTATGACGAGATTGTTAAGATTCTTTTTGTAAATTTTCTCCAAAAAAGCAAA
20 GGAACCTTGCGCACAAAATTGTAGAAATGACTCCTAAACACAACCAAATGGATGAGGG
GTTTATAACTTCTTATCTAAACCAAATAATGATTTAGAATTTGAAAAACAAACAGTT
GAGGTACTTCGAGCTTTGGGTTTTGAAACGGTCCTTCAACCTAAGCCTGTTGTAAAG
GAGTAAGAAGTAGTATTGAAATATTAATTCATCTAGATGCGCAAAATATTGCTATTAT
AGATGCAAAAACTACAAAAAGAAATTCCTCTAACAGCAAACTTGGCCAATTATAT
25 GGCTACTGAATATATCCCCATATATCAAGGCTATGATGGAAAAAATGTTACACATTTT
GGATATATTACAGCTTCTAAAATTGGCGGTAGTAGCAATCTAAAAAATAGTACAT
ACAGCTAGAACATATAATGATAAATCCGAGTTAATGGTTTTTTAATTTTCAGCGCCTG
CTTTACTAGGTCTTCTAGATTATTGTATAGAAAATAGTATTGATAATTCTCGAAGAAA
ACAATATTTCTTAACACTATTAAATGATAATAAAGCATATGAAACCTACAGTGAAGTA
30 TCACAACGATTAAATTTATCTTAA

SEQ ID NO: 8

ATGAAAAAATTCGTAATAGTTTAATTGTAATAATGAGTATTTTTGCGTTAACATTTG
GGATGATACCATCAGGAGTTGCTAATGCACAAGAGCCTAACGAAGAGTCTGATTTTA
35 AAATCACAGACCATGTTGTAACCTGACAATAAAGAGTCTTTTACAATTGCTATGAACGG
TGAGGAAGTGGATTATAAGTCGACTAAAACGGAAGATTCATTCGAAATTGAACTGT
AGAAGAAAATAATGAAGTACATACCTTTACATATACTAAAGGTGACGATTACATGAT

GATAGACGGCGAAAAAGTATCTGTTGAAATTGATACAGAAGTAGAAGCAGAATTAAC
AGATTCTTCTGCACCAAACTATTAAAAGCTTCGGCATGGACACCTAAGTATATGTCTG
ACAGGATCTATAAAGATAAGTAAGTCAGTAAAAACATTAGGAACTCTGGTTGGAGTA
ATTGGGGGAGCTATTTCTATTGCTGCACTATCAGGGGTGGCAATTGCAACATCAACAA
5 TTGCAAGTACAGTAGGACACTGGGCTAGTGCAGTAGGTTTAGGCACTATCGCTGCGG
GTTACGCATTTAATGGTAAAGTTCAATTTAAGCAGTATCGTACAACAGGATTAGTGCC
AACAAATTATGGCAAAAGATTATATGCTTTTAGATTTCAAGATGTGCGTGTTATAGGT
ACGCTTAAAGGCAAAAAAATAGATAAACTGAAAAATGTAGGAAATTGGTTCTTT
AATTCTAAACCGTATTAA

10

SEQ ID NO: 9

ATGAGTAAATGGATTTCTACTTCTCTAGGTGAGGTAGCAAAAATAATCACTGGCCCAT
TCGGAACCCAACTTCACAAGTCAGATTATGTTGACTCTGGAGTTGCAGTTATAATGCC
TCAAAATATAGGTTCTAGACAAGTTAATTATGAGAAGATTTCTTACATTTCTGAGGAG
15 TTTGCAACGACTCTAAGGCGATACAAAGTATTAACAAACGATATTGTTTTTGCTCGTC
GAGGTGATGTTGAAAAACATGCATTTATCACAGAATCTGAGGAAGGTGAACTCTGCG
GAACTGGATGTTTTCTCGTACGATTCACAAGCGAACATGTTTTACCAGAATTTATTTCT
TTGATTTTAAGTACACCTTTTCGTGAAAAAATGGCTAGTCTTAAATGCTGTCGGTTCAA
ATATGCCAAATTTGAATACTGAAATATTAACAAATGTTTCTTAAATTTCCAGATTT
20 ATCTACTCAACAAAAGATTTTATCAACTATTTTCATCGGTTGAATATAAAATTAGAATC
AATACAAAGATAAATACTAATTTATTAGACATGGCTAAGGCAATTTACATGCATTCTT
TTTTTGAAAAACATGAGAATGCAAAGATTTTCAGATATACTTCTGGAAAAACAGTAAGTC
CAACATCCAAGTTGGTGAAGCAAGAGAGGCTCGCGGCGATTATCCATTTTTTACAAGT
GGTGAACAATTTATGAATGGGATAATTATCTTGTCAAAGATAGAAATATTTATCTTA
25 ATACTGGTGGAAATGCCGATGTCAAATTCTATATTGGTAAAGCCGCATATTCCACTGA
TACATGGTGCATTTTCAGCTAAAAATGATTTTACAGACTATCTCTATCTATTCCTTGATG
CAATAAGACCTGAATTAAATCAGAAATCTTTCAAGGTACCGGGTTAAACACCTACA
GAAAGCATTAGTTAAAGATAAAGAAATATACCTTCCTTCCAAAGAAATACTAACAGA
ATTTAACTCTATTGTGAAACCGATGATGGAACAGGTATCCTTCAATACTCGTAATAAC
30 CAATACTTATCTGACCTACGTGATTGGCTATTACTTGTCTTGATGAACGAAGTAGCAA
CAATAGAATAG

SEQ ID NO: 10

ATGACCAAGAAAACTACCATTTTCGCATCCCGTGATAAAATCATATGTTGATAACTTTA
35 TTTCTGAACATGAGATATCAAAATCAAAAGCCAAAAACCAGCATGGTATATTTGAAC
AATATATTAATGATTTAATTTTATCAATTTATTCAAACGATCCTAGTGTAACCTTATCAA
GATATGGAGACAGGTTCTGCATTTGGAATTGATGGAGTTGCTATATTTGTTTCAGATC

GAGTTGCAACTAGTACGGAAGATGTTGATTCTATAATTGAAGGAGTAAAAAAATTTG
AAGTAAGCTTTTTCTTTACACAATCAAAAACAACAGATCAATTTGATCGAACAGATAT
TTCTGATTTTTTTTACAGCTGTTAGACGTTTTTTTCAATTTTAATAAATGTGAAATACCTG
AACTTAGAGAATATTGGGATGTGCGCAAATATATCTACTCTAAAGGTTCAAATTCAA
5 ACGGCAACCAAGTTTAAATATGAAATTCATTACTTTATCTCCTAAAGAAATAGATTTG
ACTGATATCCATATGAAATCAGAAATAGATTTGGGATTGAATGATTTAAATGACATGA
GCCTTTTCAATGAAGTGAAAGTTCCTGACTTTTTTAGGAATCAAAGATATTATGTCATT
ACATGAGAAAAACACTTCCGGCTTAGAAGTTTCTGTGACTTTAACAAAACACTACCCGTA
CCATATCCTAAGGATACTCATAATAAAATAAAAAATGGGTATTATGGATTAATTAAC
10 TTGAAGAATTTATACTTTTACTAACAGATGAAGTTAATAATGAAAAGATTTTACGAAA
AGGTATCTTCAATGATAACATTCGTTACTATCTTGGCTCATCAGAAAAAATAGAGGTC
AATACTTCAATGAAAAATCAGCTGTTAGGTACAGAAGATTATTTGTTTGGATTATTAA
ATAATGGTATTACTATAATTGCAGATGAGATTTTCAATTAAGTTCTGATGACTTAACACTT
ACTAATTACCAAATAGTAAATGGGTGCCAAACAAGTAATGTTATATTTGAATGTTTAG
15 AGTCTATTGAGCAATCTAGTGACATATATATTCCTATTAGGTTGATTTCAACAGAGGA
CGAAGACACAAAGCACTCCATTATAAAAGCTACTAATAGTCAAACACAATTAAGCC
AGAACAGTTGGTTGCTTTATCAGCAATTCAGAAAGCTATTGAGCAGTACTATAATACA
AAGGCAAAAGGAACTCATGCCCTTTATACTATGAACGTAGAACTGAACAATACCGA
GATGAAAATATACCAAAGACAAAAATAATCAATATACCATTTCAAATCAAGGCTACT
20 TCTGCTTTATTTTTTTGATTTACCTCATGAAGTTTCTGGTCAATATGGTAAAGTTGTAA
ACAAACAGGTGATTTGTTATTTAAAGATACGCATCTATCTTTTCTTAACTCTTATTACG
TAAGTGGTCTTGCTTGGTATAAAGTTGAAAGATTTGTACTGAATAATGAAGATGGCAA
AAGATTCAGAAGAGCTAGATGGCACATTATAATGATTTTAAATATCTCTGCTTCCCT
ATGGACAAAATAAAAATTTCTATTGATAAGCAATCTGAACAAAATAGTATGATATTA
25 GAAAAAATACTACTTGATGATGAAAAACAAATGAACTTTTGAAAAAGGCAATAGAT
ATTATTGAGAAAAGTTTAAGTCAAATGAATCTATTGAAGAAGTATTAGCGGATAGA
AAAACATTTGAAAGAAAAGAACTACAACCACTATTATTGAATATGTGAAAACAAAT
AACATAACTAA

30 SEQ ID NO: 11
ATGGGTGTAAAATATGTAGAAATCGAAAAAATTGATAACTATATTGAAAATCCTAGG
CATGAAGTAGGTCTGAATGAATTAGATACTTTAAAAAAGTTATTTGATGTTTCTGGAT
ATCAAAATATGATTAATTTGGCACAAGATATCTATACTAATGGTTTGGTAAATGCAAG
TCTAGTTACAATTGTAAAACCTCAATAATGCCGATAGATATACTGTATATGAAGGTAAT
35 AGACGAGTTGCATGTATAAACTAATTTTACATCCAGAAAAATTTAGTTTTTTACCAA
AGAACCAAATTGATCGAATCAAAAAAATGAAAAGTGACACTCCTTCAAAAATAAATC
TCTCTCAAATTGAATGCTTAATAACAGATGAAGAGGATGCTTTTTTTCATTATGAGGCG

TATACACTCAGGAGAAGATAAAAGGTAGGGGTCTTAAATCCTGGAATACTAAGGAACA
AGAAATCTTTAACTTCGAACTAATCCAAAAACAGTACATCTATTGCAAAGATTATT
AGTGACAAATATGAAGAATTTTTTAAAGAAGATATTCAAGAAGAAATGGCATATACT
AATATCCAAAGATTATTTAATAATTTGGAAGTGAGAGAGTCACTAGGCATTGAAAAA
5 GACAATATAGATTCTTTTTTCATGTGAAAGATTATATTTAATTAAAGGTGTTATTGAAA
AAGTAAACCAGATTGCTAAAATTGAAAATTTATCTATTTCTCGTAAATTTAATAAAAAG
GAAAATAATTGAACAAATAGTAATACCCATTATAGAAGAATTAAAATCTAAACAGTT
GGAAAGTAAAGCAATAAATTTCTATACATATTGAACAAAAAAATAAAAAGTAATAAATT
ATCAAATTCTAAAGAACAACAAACACAAAATAAAGAAGCAACTTCAAACAATGGAAT
10 TAGTGTACGAGAAGATACTGGTTTGAAAACCTGAATATAAAAGCATAGAATCTATATC
ATCCACTCTAAAACCAAAATTACCCCCAAAAATAGGGAGCGGAAAACCTTCTAAGTAG
TACTATGCCCTTTACAAATTTATATCATTCTAATGTGCGTATAAATACTCTAGTAATTG
AGATTAATCAGATTAGATATAAGGATTTTCGTTTAGCTACACAATTCTTATTAAGGTCT
CTTATGGAAGTATATGGACATGAATATATTGATTACTTTGCAAATTTAGACTATCAAG
15 ATGTCCAGAAAATGAAGGCTATAGCAAAAGACCGTATAAAACGAAATCAAACAGTAA
ATGAAATTTATTTCAGAGTTTATTAGTAACCACATAACTAAACATTTTCCTAAGTATGC
CGAACAAGCCGAATTAATTAATATATCATTATCAAAAAACAACAATAGTTCTCTAATG
AAAATTTTGAATTTTATGGTTCATTCTCAAAATCATATTCCTGATAGTATCGAATTAAT
AGAAGCTTGGTACAAAGTTAAATCTATCGTTGAAGCAATTGATAATATATTGCATGAG
20 GTTAAGCATAACTACTAA

SEQ ID NO: 12

GTGTTACAAAGAAGATTTTGGGGTATTTTTTGTGTTTGCCATATTCTTGTTTTTATTTCCA
ACTATTGGTTCTGCCGAAACATCTGGTGACTATGAATATACAATAAATGGAAATGAAG
25 CTACCATTACTGATTATACAGGACAGTCTACTGATATTACCATTCTACAACACTTGGT
CCTAATAATGAATATACCGTTACTGCTATCGGGAATGGCGCATTCAAATCCAAGAGAT
TAACTAATGTAACGATTCTTAATACAGTTATAACTATTGGCGATGGTGCTTTTACCATC
AATTCATTGAACAACACTAGTCTTACCCAACCTCAGTGCAGACTATAGGGAGGAACTCTT
TTAGTGTCAATAAATTAGAAAAAATAACTTATTCTACTGCTTTAAAAAATATTCCTTCC
30 CAAGCATTTTTAGCTAATAATTTAAAAACAGTGACTACACCAGCCACTGTTGAAAGTA
TTGATGCTTCTGCCTTTGAAAATAATTTTATTACTAATATTACAATTCAAACCCAAAT
CTTCAAATGGCTTATCAAGCTTTTGCCGCTCAAACAGTTTTAAGTACACTCATTGTACC
AAGCAACCACATCCTACCTATAGAAAATTACATTCAGTTTCAAGATGCTTCGGCTCAT
TTGACTACAGATAATTTATTCATAACTGATTTAGCGAATGGTATTACGTATAATCAAG
35 CCGAAAAAGCATTAACCTTTTTCAGCAGAGCCACTCGAATCTACTTTTTCACTTTTCACT
GGGACAAATCGATTTGATTCTTACTATGATATTTCTGAATATGGTCCTTCTGGAAAGC
CATTTATCTACTTCAAGTATACTAAACCAGTCCTAGTTTCCTATAAAGATGCGTCTGGG

AATGAATTAGCTACTTCTACTAGATTAGATGGTAGTATTGGTGAAAATTACGTCCTA
CTCCTAAAATAATAGATGGCTATACTTTAAAAGAAACACCCGGAAATGCGACAGGAC
AATTTTCTGAAACACTGCAAAATGTAACGTATATTTATGAAAAAACTGCTGTCCAAAA
TGGGACAGTCACTGTAAAATATCAAGATGAATCGGGTAAAACACTAGCAAAAGATAC
5 TGTTTTAACCGGTGAAGTAAATAATACCTACCAAACATAAAAGCAAAGACATAGCAGG
CTATAAACTTCAAAAAGTGGAAGGAAATGAATCGGGTACTTTTAGCACAAACCCTGC
AACTGTTACCTATATTTATGAAAAAATAGCAAACAGTGATAATACAAATACCAATGG
AGAAATGACCGACAATACTACACTGAGTACTAATGATACTGTGATTTTCATCCGAAGCA
ACAAAAAAAGTTGATAAAAACACCTCTAATATCCTCCCACTACTGGTGATTCAAAA
10 GATGCTCTCTTTTTTGCCTAGGAAGTTTATTGACGTTGTTATCTACTAGCTTCTTTTTT
TTTAAAAGAAGTTAA

SEQ ID NO: 13

ATGGCAACGCAAAAGAATTTAGTTGATTTAGCAACAAAAGCTTATATTTACGGTTTTTC
15 CTTTCATTTTTTAATACACAACAAATCGAGCGTTATGTAACGGTAGGGATTGGTGGAAC
AAAGCAAGTTCCTTTCAATAATTTTACTCATGCATCTCGCTTGGCAGAACCGTCTGAT
AAATTTGTCAGTGTGAACAACGATACTATTTATTCTAATGCACCAATTGATGTTAGTG
CCGGACCGGTCGTTTTAAGTGTACCCGATACAAGTGGTCGTTATTATGTACTTCAGTTT
GTTGATGCATGGTCAAACAATTTTGCTTATGTTGGAAAACGTGCAACGGGGACTTCAG
20 CTGGCAAATTTCTTTTAACTCCTCCAAATTGGAACGGTGATGTTTCTGCTGATATGATA
GAAATTAAATTTCTACTAATATAGGGATTATTATTGGCCGACTTGCTTGTGACGGTG
AAGCTGATTTACCAACTGTAAGAGAATTACAAGAACAACCTTAAGATAACTCCTTTAAA
TGAAGGGAAAGAAGTGGATGGTTTTCTGAATATGATCGCTCACTAGGAAAAGAGCT
AGCTTTCTTTGAACAATTACGAGTTTATATGGCTCAATTTCCACCAGCAGAAAGAGAT
25 TTGGTTAAGCAAGAAAGTTTTGCGCCAATTGGTTTGATGGAAAAGGGGTATCTCCTT
ATAGTAACCCGAGTGAAGAATTAATAAATGCCCCTTATAGAGGGAGCAAAAGCAGGTT
TAGCGAATATCAAAAAAGCAACAACCAATTTTAAATCAGAAAATGGCTGGGGATTAA
CACACATTTATTTGATTATAATGCTGATTTTTTTGAAATAGGAACGAAAAAATCTTT
AGACTGGGTAATTGAGGACCGTGAAGAAGCATAACATAATTCGTGCAGTTTCAGCTATT
30 ACGGCATTATGGGGAAACCATGGCTATGAAGCGGTTTATTTGATGACATGGACAGAC
ACAGACGGTAATGCCTTAAATGGAAAAAATAAATATACATTGGAGCTAAATCCAATT
CCGCCAGTTGACTCATTTTGGTCGATTACGATGTATGACTTGCCTGAATACTTTTTATG
TGAGAACCCGATTAATCGTTACTCGATTGGAGACCGTACGCCAGGTATACAATATAAT
GATGATGGCGGTATCACTATAGTGGTTTCGAAAGATGAGCCAACTGACCCGAAAGAA
35 CGTGCGAATTGGTTGCCAGCTCCTGATGCTGAGTTCAGACCAATGTTCCGCTTATATA
ATCCGCAACAACCACTCGTGGATGGAAGCTATCATTTTCCGGGATTGAAAAAAAGTA
A

SEQ ID NO: 14

ATGAGCAACAGTAAATTTATTATTACAGAATTATACTGGGAAAGAATTCAACTACATG
TAAAGGGTAAAATAGAAAATATTTTCGTTAGAGAACTATACGTTTTTCATTTAGGACTTT
5 AACAGATGAATTTTCATTTTTTCACCAACGGGAATCAAATTGGAAGGAAACACATTTGAT
CTTCGTTTCAATATTGCTATTTTAAATGATGGGAATTATTTACCAAGTGGGGATTATCT
CTTATCCTTATATAACAGTGAAACAGCGGATGAAATTGTTGCCCAACCGGCTGCTGAA
CTATATAAATTTCCAGAAGATGAAGATCTTTTAGAAGAACTAAACGAAGCTAAAACC
GAAAACGAAAAAACAATGTGCTTTTAGCAGGTTTAAGAAAAGAATTTAAACGCGGT
10 GGGGCGAATCTCAAGTATACGTATAAAGTCACCCCAATGATTTCTGCAGATGTAAATG
AATTTGTTTTCTCTGTTTCATTTACAATCCCTGTACCAAAACCAACAAAGTGGCAAGTA
TTCAAAAATAAAATTAAGAAATGTATCATACTTTCAGTTTTAATTTTAGAACTGGTTT
GTTTAAATCTATTTTCTATACCTCACAAGCCCTAGTACCTAAAAACGGAAAACGAATT
CTTTTTTCTTCAGATTCCCGTGCAGAAATTGGTGGTAATTTTGAGTTCGTTCTAAATAA
15 AATGAAAGAGATGGGTATTGATAAAGATTATAAAATCAAATGACCTTTAAACCCAA
CATTCGTTTGAGAAGAGCTTTTATTGAGAAATTTAGATTTCCATTTTTGCTAGGGAGTT
CAGATATTATTTTATTGATGATTATCATCCGATGATTTATACAATTGACTTCAGCGAA
AAAACACAAGTAATTCAATTATGGCATGCATGTGGGGCTTTTAAACAGTTGGATTCA
GTCGTTCAAGTAAGCAAGGCGGGCCGTTCTTTGATGATAGAGCACATCGTAATTATAC
20 ACGTGCTATTGTTGCTTCAGATACAGACATACCATTTTATGCAGAAGCTTTTGGGATA
AAAGAATCTTCTATTTTACCAACTGGTGTACCAAGAACAGATATTTTCTTTGATCCAG
AGTACAAAAGAAATATTGTTACTACGATGGAGCAGTTGTTCCAGAACTCAAGGCA
AACAAAGTTATTTTATTTGCTCCAACCTTTCCGTGGAAATGGTGCGAATCAAGCTCATTA
CCCATATTTTAAAATTAATCTGGCTAAGTTTGCTGAATACTGTCGAAAAAATAATTCTG
25 GTCGTAATCTTTAAATGCATCCGTTTATTA AAAATGAATTTATTATTCCAGAACAAT
ATGCAGATGTTTTTCATTGATGCTAGTTCTTATCGTGAAGTAAATGATATTCTATTCATT
ACGGATATATTGATTACGGATTATTCATCTGTTATTTTCGAATTCTCTACCTTACAACG
CAAAATGCTGTTCTATGCATTTGACTTGGAAGATTATGTTTCTACGCGTGACTTTTATG
AAGAATATGAAGGTTTTGTGCCAGGGAAAATTGTATATGATTTCGAAGCATTAAATTAA
30 GCGCTTGAAATGAATGATTTTGAACAAGAAAAGGTAAAACCATTTTTAGATAAGCA
CTTTAAATATCAAGATACTAATTCTGCAAAACGAATTGTGGAAGAAATCTTTAAAAAA
TAA

SEQ ID NO: 15

35 ATGAAAAAGCTTAGAATATTTACTTTGATGCTTGCTTTTGCTCTATTTTTATTAGGTGG
AGGAATTGTTGCACAAGCAGCAGAGGAAGCCCAATCGATGAAAAGATAGTTGGGGA
AACGGTAACAAATGACGGAGAAGAGTTTATAGTCGATGAGATTAGTGATTTAGAAGA

TGTTAATTCAACAACGGGGACGTTAGATACATCTGATGAAGTTGAGGTTGATTTATCA
 GGATTAAGTATTGAAAATGAGGAAGCCGTTCTAACCCCAGGTTTAAAAACGATTTTTG
 GCGGAGATGGTAGAAAACCTTGTAACAAATACAACTCAATATCCATATTCAACGAGCG
 CTTACCTTGTAATGGAATTTCCAAATGGAAAACTTATATAGGTAGTGGACAATTGAT
 5 TGGTGAAGATTCTGTACTTACAGCCGCTCATTGTTTATATGGTAAAAAAGATGGTGGA
 TGGGCAAAAAAAGTGACTGTATATCCTGGATATAATGGCACGAAAGCTCCTTTTGG
 ACAGCAAAAGCAAGAAAAATGTATGTTCCAAAAGAATGGACAAAAAAGAACCTTCT
 ACAGAAGATTATGGTGTATTAAATTAGATAAAAAATATTGGGACAAAAACTGGAACA
 ATGGGGTTAACAATAACATCTGGTGCAATTACTATTAGTGGTTATCATGGTGACA
 10 AAAAAGGGAAATTGTACACTCAAACCTGGAAATATCTCTCAAGTCACTGCAAATAATG
 TTTTTTATAGATTAGATACAACAGGTGGTAGTAGTGGTAGTGGTGGTTTATAATTCTAA
 AAAACAGATTTTAGCAGTAAACGCATATGAATATTTAAATGGTACCGGGGACAACCTT
 GGTACAAGAATAACAAAAGAAAACTAAATAATTTTATACTTGGGCGTTTGACAAT
 AATCTTTCTGTAAGCAAACAAAAAGGGATAAATTACGAGCTCCACGTCCAAAGTAAG
 15 GGATGGATGGGAAACGTTGCTAATAGTATGACTTCGGGCACAGTTGGCCTTGGCTTAC
 GAGCAGAAGCAATGAAAATTAGTTTATCAGGGATGCCGTATTCTGGAGATATTCAAT
 ATCGCTCACATGTACAAGGTAGTGGATGGCAAGGTTGGTTGAAAAATGGGGAAATTT
 CTGGAACCTCGTGGACAGTCAAAACGTCTGGAAGCTTTCCAAGTAAAATTAAGTGGAA
 ATATGGCTAAAAATTATAGTGTGCAGTATAGAGCGCATGTCCAAAATAAAGGTTGGC
 20 AAAGTTGGGTAAAGATGGAGCAACAGCAGGAACAACAGGAGAAAGTTTAAGAATA
 GAAGCTGTACAAATGCGACTAGTTGCAAAATAA

The sequences of SEQ ID NOs: 61-181 are shown in the sequence listing, which is filed together with the application (ST25.txt file), and which is herein incorporated by reference

25

Example 3: Multiplex PCR method to identify major clones of *L. monocytogenes*

Multiplex primers were then carefully designed (*cf.* Tables 6, 7 and 8 below) to produce amplification products with different sizes and to amplify DNA under a unique temperature.

30 Target genes were grouped into three 'clonogrouping' multiplex PCR assays. These clonogrouping multiplex PCR assays can be implemented on their own, or can be used downstream of the widely used PCR serogrouping assay. First, the IVb clonogrouping PCR assay was designed to identify IVb clones CC1, CC2, CC4 and CC6, or any other IVb isolates. Second, clonogrouping PCR IIb was designed to identify IIb clones CC3 and CC5, or any other serogroup IIb isolates. Third,

35 clonogrouping PCR IIaIIc was designed to identify either serogroup IIa clones CC7, CC8, CC121 and CC155, or clone CC9, most isolates of which are of serogroup IIc.

Expected amplification results are given in Table 9 below.

Table 6:

Target and primers for selected CLONE-SPECIFIC GENES					
SEQ ID NO: of the genes	Clone specificity	SEQ ID NO:			Multiplex grouping
		Selected target	Forward primer	Reverse primer	
1	CC1	16	17	18	Multiplex PCR IVb of the application
2	CC2	19	20	21	
3	CC4	22	23	24	
4	CC6	25	26	27	
5	CC3	28	29	30	Multiplex PCR I Ib of the application
6	CC5	31	32	33	
7	CC121	34	35	36	Multiplex PCR IIaIIc of the application
8	CC155	37	38	39	
9	CC7	40	41	42	
10	CC8 and/or (and) CC16	43	44	45	
11	CC9	46	47	48	

The primer pair of SEQ ID NO: 47 and SEQ ID NO: 48 has been disclosed in Doumith *et al.* 2004.

5

Table 7:

Targets and primers for selected CONTROL GENES					
SEQ ID NO: of control genes	Lineage/subgroup specificity	SEQ ID NO:			Suitable in multiplex with
		Selected target	Forward primer	Reverse primer	
12	Lineage I	49	50	51	Multiplex PCR IVb and I Ib of the application
13	Lineage II	52	53	54	Multiplex PCR IIaIIc of the application
14	Serogroup IIb and lineage II	55	56	57	Multiplex PCR I Ib of the

					application
15	Serogroup IVb (lineage I)	58	59	60	Multiplex PCR IVb of the application

Sequences SEQ ID NOs: 16-60 are as follows (in the 5'-3' orientation):

SEQ ID NO: 16

5 TCAGCAACTCAAACGGAAGAATCGATGGAGTACACTATAGTATAGCTGATATACCAA
TAGAAAAAACTTTTGAATTGAGAAGTGAGGGTATTTACACTTTTCCTGAACTACCTCT
TTACTATCTAACCACAAAAAATGATTATATTGAAAAATATTTGGATCGATCACCAATT
AATTTAGAGGATGATAAAAGCTTTATCCCTAACGAAAGAAATAACAAGCTTATCTGC
ATGGAAAAGCATATTGATTTATCATTTTTTAAGTACGCTACCATTATCAACTTTTGAGTA
10 TTCTACAAAACGTGAAGAATTTTTAAATATGGTTAATACATTTTATGATACTTATTACG
AAGATGATTATCTTATGACGCCGACATTTATGATTAATAAAGTCGGTTCGTGGGAAG

SEQ ID NO: 17

TCAGCAACTCAAACGGAAGA

SEQ ID NO: 18

15 CTTCCACGAACCGACTTTA

SEQ ID NO: 19

GCATTTTCCAAAAGCAGAGCTTGAGCGTTTATTGGAAGGAAATCTTTAAAGTTTTTGA
GAAATTAAGAATCAGAACCTATCGGACAAGATGATATAAATAAAATGAGAAGAA
20 ATCTTTCCCAAGAGCAAGAAATTATTGAAACAGCTTTCTCTAGTGATGCTGATTTAAT
GGGTTATTGGTTAATTGAACAACCTTCAAAGGAACTTCAAATCGTAGTAGTATTTTT
TATGATGATGAGATACTTTCAATTAATTTGAAATCTGAAATGATTCCATTAATTGTTAA
ACTAATGACGGATGAACTAGATATATTTATTGGTCTTTTAAAGAAATTATCTCTGAT
GAGCTGATAAGAATTATATATGTATCTTCTGAATGACAAAATGTTGTTGGATAATA
25 AGTCAATATTAGATAATTGTCCTGACTTATTACTAGAATCTCATCCTTTCCAAAGAATT
TTAGTAAGTATTGATAATGAGGTTGGATTGAATGATTCTATTAATAAATAAGTTGATAA
CTAAAATTGATTTTTTCCAATACTACCTTTGGAAGCGAGCTAAA

SEQ ID NO: 20

GCATTTTCCAAAAGCAGAGC

30 SEQ ID NO: 21

TTTAGCTCGCTTCCAAAGGT

SEQ ID NO: 22

GGGGGTAAACCACAAGTTTTATCCGCATTTAGAGACTGTAAAGAACATGTTTCTAGCA
ATAATAATATAAAAAACATATATCTTTTAGATGGTGATTTTGATCAATATTTGGATAA
TATAGTAATGGAATCACATCCACATATTATTTATTTGCAATCATATAATATTGAAAATT
ATTTTTTTGAATGAAGAATCGGTAGTAACCTTTTATGCAAGGAAAAATGAAAATGCTGAA
5 GAATGATGTAAGGACTAAAATAGCTTATTCTGAATGGCTTTCTAATACATTATACAAT
ATAGAAAACATTTTTATTTTATATTGTATAATTCAAGAAAAGGAATTAGGGATTCCTA
ATGTAGGGGATTCTGAATATAAAGTAATTAATATGATCGATGGATCTATCAATATGAC
GAGATATGAGGATTTGAAAAAGCAAATAAGTAATTGTACAGTAAACATAGATTTAGT
AGAGCAAGAAATGAAAGCAAAAATTCACAATATAAATCCTAATGTGTTTGATTTGATT
10 TGTGGTAAACATTTGATTTGTAGTTTGTACCGTCATCTGTTAAAAATAACTAAAGGTTT
CAGCTATGATGAATTTAGATGGCATTTAGTTCAAAATGTTG

SEQ ID NO: 23

GGGGGTAAACCACAAGTTTT

SEQ ID NO: 24

15 CAACATTTTGAACATAAATGCCATC

SEQ ID NO: 25

TGAGGCTATCGAAATTGCTCGCTATTTTGATACAATAGGTGACAGTCAATTATCGGAA
TACATCATGAGTTTGTTATCTACAGCAAATACCTTTGTGCCGCAAGGTGAGGCTTTTG
20 AGAACTTATTTTTCAAAAAAATAGAACCTAATGTAGAACCATTACCACTCCCTAAAGC
AATTAGTGAAGATATTCGAGGTATTATAAATGCTGTAAATCATCATGTAGGAATAAAT
AAATTTCTCTTCGAGGGTGCACCTGGAAGTGGAAAGACAGAAACGGTTAAACAGATT
GCTCGATTATTAGAACGCCAACTATATATGGTAGAATTTAGCGAATTAATTGATAGTA
AAATGGGGCAAACAGCTAAGAACATTGTAGCAGTGTTCAACGAAATAAATCGTGTAT
25 CAAATCCATCAAATTATTAATACTTTTCGATGAAATAGATGCTATAGCCCTAGATAG
AGTAAACTCTAATGACTTAAGAGAAATGGGACGGGTAACATCTACAATCTTGAAAGA
GTTGGATAAGTTAAATGAAGAAGTTGTTCTTATTGCCACTACTAATTTGTATGAAAAA
TTTGATAGAGCCTTAATAAGAAGATTTGATTCAGTAATTAATTTTAATAGATATGAAA
AGGAAGATTTAGTAGAAATTTTCAGAAATCATATTGAATTCATTATTGAAAAAATTTAA
30 AGATGCTGGACGAGATATGAAGTTATTTAAAAAAGTAATCAGAAACATGGATGAAAT
TCCGAATCCTGGAG

SEQ ID NO: 26

TGAGGCTATCGAAATTGCTC

SEQ ID NO: 27

35 CTCCAGGATTCGGAATTTCA

SEQ ID NO: 28

TCGATTTGCGAATGAAAGAAAATATTGATGATCTTAGCATTGAAGAACTTGCTGATTT
AGAAATTGATTTAGATGTTTTGTTTGATAATGTCAGAGGGTATCTAGGAAACACAAAT
TATAATAAAAAAATTCTTGAAACTTTGAAAAAGAACCTGGAAAATTCTTTTTATTTA
ACAATGGTATAACAATTACTGCAGATGATATTAATGTGAAAGAAGTTAGACTGGGTA
5 AGGCTAGTCGAATTAAGCTAGATCGGTTTCAAGTTGTCAATGGGGGACAATCCTTACG
AACTATATATAAATACAAAGATCAAAAAGACTCACCAATTGAAAATTTAGCTAAGGC
AAGTGTATTAGTGAGATTTTTTAAAACAGGGCAAGAAGAGGGATTAATTAATAAGGT
ATCAGAATATACCAATAGTCAGAATGCTATCTCAGCTATTGATTTAAGGACAGTTGAT
CAATTACAATTA AAAATTGAGAAGCACTTAGAACTCAAGGGAATTCAATATATAAGA
10 AAGAGAAGTTCTGATAAACTAAGTTGCAACTAGAAAATTCTATTACAATGGAAAAA
CTAGGCCAATTGTTATTTGCATACGCGGGTT

SEQ ID NO: 29

TCGATTTGCGAATGAAAGAA

SEQ ID NO: 30

15 AACCCGCGTATGCAAATAAC

SEQ ID NO: 31

TGCCACCTTGGTGTTAATGAAATAGATGAAGATAATATGGAAGACTTTGTTAAGACTT
ATAAACAATTAATAAAGAAAGGCGAATAAAACAATGCTTATATCCAGAAAAATCTA
AATGCAGTGATAAAATAATTAGAGCGCATTCTATACAAAATAGTAAATAATTCAAC
20 GAATATCTGAGAATGGGAATGTTTATATGAACAAAGCAAAACCAAATAATCCATTG
CTGTATTTACTAAGTGGGGGCGTAAAGAAGCAACGACATTTACTGGATTTTGTGATTA
TCATGATAAAGTTGTATTCCAGCCAATTGAAGATAGAGACTTTGAGTTAAGTAATGAA
CAGATCTTTTTGCATACTTATAGATGTTTGGTAGCTAACATGCATGATAAGCAAGAAC
AAGTTAAGTTAGGGAAATCAACTACTGAACTTTTTTCATTATCCAGTGAATCTAGTGT
25 TGGATTATTGGGCTTAGGAAATGGCT

SEQ ID NO: 32

TGCCACCTTGGTGTTAATGA

SEQ ID NO: 33

AGCCATTCCTAAGCCCAAT

30 SEQ ID NO: 34

TTTGAAACGGTCCTTCAACCTAAGCCTGTTGTAAAGGAGTAAGAAGTAGTATTGAAA
TATTAATTCATCTAGATGCGCAAAATATTGCTATTATAGATGCAAAAAACTACAAAAA
GAAATTCACCTCTAACAGCAAACTTGGCCAATTATATGGCTACTGAATATATCCCCATA
TATCAAGGCTATGATGGAAAAAATGTTACACATTTTGGATATATTACAGCTTCTAAAA
35 TTGGCGGTAGTAGCAATCTAAAAAAATAGTACATACAGCTAGAACATATAATGATA
AATCCGAGTTAATGGTTTTTTAATTCAGCGCCTGCT

SEQ ID NO: 35

TTTGAAACGGTCCTTCAACC

SEQ ID NO: 36

AGCAGGCGCTGAAATTAAAA

5 SEQ ID NO: 37

TGCGTTAACATTTGGGATGATACCATCAGGAGTTGCTAATGCACAAGAGCCTAACGA
AGAGTCTGATTTTAAAATCACAGACCATGTTGTAAGTACATAAAGAGTCTTTTACA
ATTGCTATGAACGGTGAGGAAGTGGATTATAAGTCGACTAAAACGGAAGATTCATTC
GAAATTGAACTGTAGAAGAAAATAATGAAGTACATACCTTTACATATACTAAAGGT
10 GACGATTACATGATGATAGACGGCGAAAAAGTATCTGTTGAAATTGATACAGAAGTA
GAAGCAGAATTAACAGATTCTTCTGCACCAAACTATTAAGCTTCGGCATGGACA
CCTAAGTATATGTCGACAGGATCTATAAAGATAAGTAAGTCAGTAAAAACATTAGGA
ACTCTGGTTGGAGTAATTGGGGGAGCTATTTCTATTGCTGCACTATCAGGGGTGGCAA
TT

15 SEQ ID NO: 38

TGCGTTAACATTTGGGATGA

SEQ ID NO: 39

AATTGCCACCCCTGATAGTG

20 SEQ ID NO: 40

AATAATCACTGGCCCATTCGGAACCCAACTTCACAAGTCAGATTATGTTGACTCTGGA
GTTGCAGTTATAATGCCTCAAAATATAGGTTCTAGACAAGTTAATTATGAGAAGATTT
CTTACATTTCTGAGGAGTTTGCAACGACTCTAAGGCGATACAAAGTATTAACAAAACGA
TATTGTTTTTGCTCGTCGAGGTGATGTTGAAAAACATGCATTTATCACAGAATCTGAG
25 GAAGGTGAACTCTGCGGAACTGGATGTTTTCTCGTACGATTCACAAGCGAACATGTTT
TACCAGAATTTATTTCTTTGATTTTAAGTACACCTTTCGTGAAAAAATGGCTAGTCTTA
AATGCTGTCGGTTCAAATATGCCAAATTTGAATACTGAAATATTAACAAAATGTTCTTA
TTAAATTTCCAGATTTATCTACTCAACAAAAGATTTTATCAACTATTTTCATCGGTTGAA
TATAAAATTAGAATCAATACAAAGATAAATACTAATTTATTAGACATGGCTAAGGCA
30 ATTTACATGCATTCTTTTTTTGGAAAACATGAGAATGCAAAGATTTTCAGATATACTTCT
GGAAAACAGTAAGTCCAACATCCAAGTTGGTGAAGCAAGAGAGGC

SEQ ID NO: 41

AATAATCACTGGCCCATTCG

SEQ ID NO: 42

35 GCCTCTCTTGCTTCACCAAC

SEQ ID NO: 43

AAATTCAAACGGCAACCAAGTTTAAATATGAAATTCATTACTTTATCTCCTAAAGAAA
TAGATTTGACTGATATCCATATGAAATCAGAAATAGATTTGGGATTGAATGATTTAAA
TGACATGAGCCTTTTCAATGAAGTGAAAGTTCCTGACTTTTTAGGAATCAAAGATATT
ATGTCATTACATGAGAAAAACACTTCCGGCTTAGAAGTTTCTGTGACTTTAACAAAAC
5 TACCCGTACCATATCCTAAGGATACTCATAATAAAATAAAAAATGGGTATTATGGATT
AATTAACTTGAAGAATTTATACTTTTACTAACAGATGAAGTTAATAATGAAAAGATT
TTACGAAAAGGTATCTTCAATGATAACATTCGTTACTATCTTGGCTCATCAGAAAAAA
TAGAGGTCAATACTTCAATGAAAAATCAGCTGTTAGGTACAGAAGATTATTTGTTTGG
ATTATTAAATAATGGTATTACTATAATTGCAGATGAGATTTCAATTAAGTTCTGATGACT
10 TAACACTTACTAATTACCAAATAGTAAATGGGTGCCAAACAAGTAATGTTATATTTGA
ATGTTTAGAGTCTATTGAGCAATCTAGTGACATATATATTCCTATTAGGTTGATTTCAA
CAGAGGACGAAGACACAAAGCACTCCATTATAAAAGCTACTAATAGTCAAACACAAT
TAAAGCCAGAACAGTTGGTTGCTTTATCAGCA

SEQ ID NO: 44

15 AAATTCAAACGGCAACCAAG

SEQ ID NO: 45

TGCTGATAAAGCAACCAACTG

SEQ ID NO: 46

20 AGGGGTCTTAAATCCTGGAATACTAAGGAACAAGAAATCTTTAACTTCGAACTAATC
CAAAAAACAGTACATCTATTGCAAAGATTATTAGTGACAAATATGAAGAATTTTTTAA
AGAAGATATTCAAGAAGAAATGGCATATACTAATATCCAAAGATTATTTAATAATTTG
GAAGTGAGAGAGTCACTAGGCATTGAAAAAGACAATATAGATTCTTTTTTCATGTGAA
AGATTATATTTAATTAAAGGTGTTATTGAAAAAGTAAACCAGATTGCTAAAATTGAAA
25 ATTTATCTATTTCTCGTAAATTTAATAAAAGGAAAATAATTGAACAAATAGTAATACC
CATTATAGAAGAATTAATAATCTAAACAGTTGGAAAGTAAAGCAATAAATTCTATACA
TATTGAACAAAAAATAAAAGTAATAAATTATCAAATTCTAAAGAACAACAAACACA
AAATAAAGAAGCAACTTCAAACAATGGAATTAGTGTACGAGAAGATACTGGTTTGAA
AACTGAATATAAAAGCATAGAATCTATATCATCCACTCTAAAACCAAATTACCCCCA
30 AAAATAGGGAGCGGAAAACCTTCTAAGTAGTACTATGCCCTTTACAAATTTATATCATT
CTAATGTGCGTATAAATACTCTAGTAATTGAGATTAATCAGATTAGATATAAGGATTT
TCGTTTAGCTACACAATTCTTATTAAGGTCTCTTATGGAAGTATATGGACATGAATAT
ATTGATTACTTTGCAAATTTAGACTATCAAGATGTCCAGAAAATGAAGGCTATAGCAA
AAGACCGTATAAAACGAAATCAAACAGTAAATGAAATTTATTCAGAGTTTATTAGTA
35 ACCACATAACTAAACATTTTCTAAGTATGCCGAACAAGCCG

SEQ ID NO: 47

AGGGGTCTTAAATCCTGGAA

SEQ ID NO: 48

CGGCTTGTTTCGGCATACTTA

SEQ ID NO: 49

5 GGTCTTCTGGAAAGCCATTTATCTACTTCAAGTATACTAAACCAGTCCTAGTTTCCTA
TAAAGATGCGTCTGGGAATGAATTAGCTACTTCTACTAGATTAGATGGTAGTATTGGT
GAAAATTACGTCACTACTCCTAAAATAATAGATGGCTATACTTTAAAAGAAACACCCG
GAAATGCGACAGGACAATT

SEQ ID NO: 50

10 GGTCTTCTGGAAAGCCATT

SEQ ID NO: 51

AATTGTCCTGTCGCATTTCC

SEQ ID NO: 52

15 CAGAAAATGGCTGGGGATTAACACAACATTTATTTGATTATAATGCTGATTTTTTTGA
AATAGGAACGAAAAAATCTTTAGACTGGGTAATTGAGGACCGTGAAGAAGCATACAT
AATTCGTGCAGTTTCAGCTATTACGGCATTATGGGGAAACCATGGCTA

SEQ ID NO: 53

CAGAAAATGGCTGGGGATTA

20 SEQ ID NO: 54

TAGCCATGGTTTCCCCATAA

SEQ ID NO: 55

CGAAGCTAAAACCGAAAACGAAAAAAACAATGTGCTTTTAGCAGGTTTAAGAAAAGA
25 ATTTAAACGCGGTGGGGCGAATCTCAAGTATACGTATAAAGTCACCCCAATGATTTCT
GCAGATGTAAATGAATTTGTTTTCTCTGTTTCATTTACAATCCCTGTACCAAAACCAAC
AAAGTGGCAAGTATTCAAAAATAAAATTAAAGAAATGTATCATACTTTTCAGTTTTAAT
TTTAGAACTGGTTTGTTTAAATCTATTTTCTATACCTCACAGCCCTAGTACCTAAAAA
CGGAAAACGAATTCTTTTTTCTTCAGATTCCCGTGCAGAAATT

30 SEQ ID NO: 56

CGAAGCTAAAACCGAAAACG

SEQ ID NO: 57

AATTTCTGCACGGGAATCTG

35 SEQ ID NO: 58

TATGACTTCGGGCACAGTTGGCCTTGGCTTACGAGCAGAAGCAATGAAAATTAGTTTA
TCAGGGATGCCGTATTCTGGAGATATTCAATATCGCTCACATGTACAAGGTAGTGGAT

GGCAAGGTTGGTTGAAAAATGGGGAAATTTCTGGAACTCGTGGACAGTCAAAACGTC
TGGAAGCTTTCCAAGTAAAATTAAGTGGAAATATGGCTAAAAATTATAGTGTGCAGTA
TAGAGCGCATGTCCAAAATAAAGGTTGGCAAAGTTGGGTAAAGATGGAGCAACAGC
AGGAACAACAGGA

5 SEQ ID NO: 59

TATGACTTCGGGCACAGTTG

SEQ ID NO: 60

TCCTGTTGTTCTGCTGTTG

Table 8. Primers used in this study.

Multiplex PCR	Gene	Putative function	Genome	Primer name ^a	Primer sequence (5' - 3')	PCR product size
PCR IVb	<i>LMOF2365_2638</i>	Putative peptidoglycan bound protein (LPXTG motif)	F2365	<i>LMOF2365_2638-F</i>	GGT CCT TCT GGA AAG CCA TT	194
				<i>LMOF2365_2638-R</i>	AAT TGT CCT GTC GCA TTT CC	
	<i>LMOF2365_1900</i>	Putative serine protease	F2365	<i>LMOF2365_1900-F</i>	TAT GAC TTC GGG CAC AGT TG	301
				<i>LMOF2365_1900-R</i>	TCC TGT TGT TCC TGC TGT TG	
	<i>LMOF2365_2702</i>	hypothetical protein	F2365	<i>LMOF2365_2702-F</i>	TCA GCA ACT CAA ACG GAA GA	405
				<i>LMOF2365_2702-R</i>	CTT CCC ACG AAC CGA CTT TA	
	<i>LMOATCC19117_2417</i>	hypothetical protein	ATCC19117	<i>LMOATCC19117_2417-F</i>	GCA TTT TCC AAA AGC AGA GC	565
				<i>LMOATCC19117_2417-R</i>	TTT AGC TCG CTT CCA AAG GT	
	<i>LMOL312_1102</i>	hypothetical protein	L312	<i>LMOL312_1102-F</i>	GGG GGT AAA CCA CAA GTT TT	622
				<i>LMOL312_1102-R</i>	CAA CAT TTT GAA CTA AAT GCC ATC	
PCR IIb	<i>LM1816_RS00050</i>	AAA ATPase family protein	H7858	<i>LM1816_RS00050-F</i>	TGA GGC TAT CGA AAT TGC TC	764
				<i>LM1816_RS00050-R</i>	CTC CAG GAT TCG GAA TTT CA	
	<i>LMOF2365_2638</i>	Putative peptidoglycan bound protein (LPXTG motif)	F2365	<i>LMOF2365_2638-F</i>	GGT CCT TCT GGA AAG CCA TT	194
				<i>LMOF2365_2638-R</i>	AAT TGT CCT GTC GCA TTT CC	
	<i>Imo1077</i>	Glycosyl transferase	EGDe	<i>Imo1077-F</i>	CGA AGC TAA AAC CGA AAA CG	334
				<i>Imo1077-R</i>	AAT TTC TGC ACG GGA ATC TG	
	<i>M637_08170</i>	conserved protein of unknown function	FSLJ2-064	<i>M637_08170-F</i>	TGC CAC CTT GGT GTT AAT GA	487
				<i>M637_08170-R</i>	AGC CAT TTC CTA AGC CCA AT	
	<i>LMOSLCC2482_0307</i>	hypothetical protein	SLCC2482	<i>LMOSLCC2482_0307-F</i>	TCG ATT TGC GAA TGA AAG AA	606
				<i>LMOSLCC2482_0307-R</i>	AAC CCG CGT ATG CAA ATA AC	

Table 8 (continued and end). Primers used in this study.

<i>lmo0525</i>	hypothetical protein	<i>EGDe</i>	<i>lmo0525-F</i>	CAG AAA ATG GCT GGG GAT TA	163
			<i>lmo0525-R</i>	TAG CCA TGG TTT CCC CAT AA	
<i>LM6179_0610</i>	Predicted protein	6179	<i>LM6179_0610 -F</i>	TTT GAA ACG GTC CTT CAA CC	327
			<i>LM6179_0610 -R</i>	AGC AGG CGC TGA AAT TAA AA	
<i>LMLG_RS02400</i>	hypothetical protein	<i>Finland 1998</i>	<i>LMLG_RS02400-F</i>	TGC GTT AAC ATT TGG GAT GA	460
			<i>LMLG_RS02400-R</i>	AAT TGC CAC CCC TGA TAG TG	
<i>LMRG_02577</i>	hypothetical protein	10403S	<i>LMRG_02577-F</i>	AAT AAT CAC TGG CCC ATT CG	627
			<i>LMRG_02577-R</i>	GCC TCT CTT GCT TCA CCA AC	
<i>LM5578_1185</i>	hypothetical protein	08-5578	<i>LM5578_1185-F</i>	AAA TTC AAA CGG CAA CCA AG	729
			<i>LM5578_1185-R</i>	TGC TGA TAA AGC AAC CAA CTG	
<i>lmo1118 #</i>	hypothetical protein	<i>EGDe</i>	<i>lmo1118-F</i>	AGG GGT CTT AAA TCC TGG AA	907
			<i>lmo1118-R</i>	CGG CTT GTT CGG CAT ACT TA	

a :F, forward; R, reverse

: Doumith *et al.*, 2004

Table 9. Expected amplification profiles.

Multiplex PCR assay	Gene target	Lineage I						Lineage II							
		IVb				IIb		IIa			IIc [#]				
		CC1	CC2	CC4	CC6	Other	CC5	CC3	Other	CC121	CC155	CC7	CC8	CC9	Other
PCR IVb	<i>LMO</i> f2365_2638	1	1	1	1	1	1	1	1						
	<i>LMO</i> f2365_1900	1	1	1	1	1									
	<i>LMO</i> f2365_2702	1													
	<i>LMO</i> ATCC19117_2417		1												
	<i>LMOL</i> 312_1102			1											
	<i>LM1816_RS00050</i>				1										
PCR IIb	<i>LMO</i> f2365_2638	1	1	1	1	1	1	1	1						
	<i>Imo</i> 1077						1	1	1	1	1	1	1	1	1
	<i>M637_08170</i>						1								
	<i>LMOSLCC2482_0307</i>							1							
PCR IIaIIc	<i>Imo</i> 0525									1	1	1	1	1	1
	<i>LM6179_0610</i>									1					
	<i>LMLG_RS02400</i>										1				
	<i>LMRG_02577</i>											1			
	<i>LM5578_1185</i>												1		
	<i>Imo</i> 1118														1

CC: clonal complex; 1: band expected to be amplified

Except EGDe, a CC9 strain which is of serogroup IIc but belongs to serotype 1/2a

A total of 282 *L. monocytogenes* strains were used to validate the method (*cf.* Table 10 below). The three multiplex PCRs were performed with an initial denaturation step at 94°C for 30 s followed by 25 cycles of 30 s at 94°C, 30 s at 55°C and 75 s at 72°C, and a final elongation step at 72°C for 10 min. Eight microliters of the PCR products were mixed with 3 µl of loading
5 buffer and separated on a 1.5% agarose gel in a TBE buffer 1x at 120 V for 90 min. The primers were concentrated at 20 µM for the PCRs IVb and I Ib and at 10 µM for the PCR IIaIc.

Among these, 185 were analyzed previously by MLST, whereas the MLST genotype of 97 remaining strains was determined in the present study. The latter strains were selected from the Collection of Listeria of Institut Pasteur (CLIP) maintained at the French NRCL and WHO
10 Collaborating Center for Listeria (Centre National de Référence des Listeria; Institut Pasteur; 25-28 rue du Docteur Roux; 75724 Paris Cedex 15; France).

DNA extractions were performed using the PROMEGA WIZARD® Genomic DNA Purification kit (PROMEGA Corporation 2800 Woods Hollow Road, Madison, WI 53711-5399 USA). PCR serogrouping and MLST were performed as previously described (Doumith *et al.*
15 2004; Ragon *et al.* 2008). Clonal complexes were defined as groups of allelic profiles sharing at least 6 out of 7 alleles with at least one other member of the group (Ragon *et al.* 2008). Allelic profiles sharing less than 6 alleles with all the other allelic profiles were designated as singletons; these groups were defined based on MLST data from this study and previous ones (Ragon *et al.* 2008; Chenal-Francisque *et al.*; 2001; Cantinelli *et al.* 2013; Haase *et al.* 2014;
20 Chenal-Francisque *et al.* 2013). Discrimination index was calculated using the web tool available at <http://darwin.phylloviz.net/ComparingPartitions>.

The 282 strains were analyzed using the three multiplex PCR assays (*cf.* Table 9 above). Figures 6A, 6B and 6C show PCR amplification patterns obtained for representatives of the eleven
25 target clones. Amplification results were highly consistent with the expected profiles of presence/absence of clone-specific genes and lineage- or serogroup-specific control genes (*cf.* Table 9 above). Overall, 222 out of 232 (95.7%) strains that belonged to target clones, were correctly identified (*cf.* Table 11 below and Figure 7): 100% of strains belonging to CC3, CC4, CC5, CC7, CC8, CC121 and CC155 were successfully identified; for other clones, correct
30 identification rates were 78.6% (CC1), 93.1% (CC2), 94.7% (CC6) and 96% (CC9). For nine strains (6 CC1 strains, 2 CC2 strains and 1 CC9 strain), identification was incomplete, being correct at the level of lineage and serogroup although the expected clone-specific PCR product was not amplified (Figure 7, Table 10 below). Only CC6 strain CLIP2009/01048 was misidentified, as CC1. Out of the 50 strains that did not belong to target clones, 47 (94%)
35 yielded the expected lineage and serogroup specific PCR fragments, and three were

misidentified as either CC1 or CC8, from which they differed by two or three MLST alleles. Therefore, in total, only four (1.4%) strains were misidentified, whereas 269 out of 282 (95.4%) were identified correctly.

- 5 In conclusion, we have developed a set of three multiplex PCRs enabling ‘clonogrouping’, *i.e.*, determining whether a strain belongs to one of the major clonal groups of *L. monocytogenes*. This novel tool can be used in a one-step strategy, which comprises the implementation of the three clonogrouping PCR assays (clonogrouping PCR assays IVb, IIb and IIaIIc) either simultaneously or sequentially.
- 10 Alternatively, this novel tool can be used in a two-step strategy, such as:
- i. implementation of the PCR serogrouping method of Doumith *et al.* 2004 to determine the PCR serogrouping profile (IVb, IIb, IIa or IIc), and
 - ii. implementation of the clonogrouping PCR assay that corresponds to the PCR serogrouping profile determined at step i. (clonogrouping PCR assay IVb, IIb or IIaIIc), thereby
- 15 allowing for identification at the MLST clone level.

Unlike MLST, our tools can provide clone identification in less than one day starting from a bacterial culture.

- 20 Based on the dataset of this study, the discriminatory power (Simpson’s index) of clonogrouping was 92.3% (CI, 91.8-92.8), whereas PCR serogrouping of Doumith *et al.* 2004 had a discrimination level of only 77.5% (CI, 76.3-78.7).

- 25 This new widely applicable tool will therefore significantly refine, beyond PCR serogrouping, the genotypic characterization of *L. monocytogenes* strains or isolates. It will thus provide improved resolution for molecular surveillance, while providing rapid information to risk managers during the investigations of outbreaks and for the control of food safety and food production environment. Finally, the wide use of this novel method should contribute to better define the distribution of major clonal groups in clinical, food and environmental sources.

Table 10:

Study order	Strain code	Original name	Lineage	ST	Clonal complex or singleton	PCR serogroup	Target category
1	LM10-01441	TS50/L.4760, FSL N3-008	I	1	CC1	IVb	CC1
2	LM10-01445	TS21/L.4486j, FSL N3-022	I	1	CC1	IVb	CC1
3	LM11-00084	CLIP2011/00084	I	413	CC1	IVb	CC1
4	LM11-00105	CLIP2011/00105	I	414	CC1	IVb	CC1
5	LM11309	11309	I	1	CC1	IVb	CC1
6	LM12980	12980	I	243	CC1	IVb	CC1
7	LM13-00428	CLIP2013/00428	I	692	CC1	IVb	CC1
8	LM13892		I	65	CC1	IVb	CC1
9	LM16335		I	79	CC1	IVb	CC1
10	LM21640		I	10	CC1	IVb	CC1
11	LM24919		I	326	CC1	IVb	CC1
12	LM25703		I	1	CC1	IVb	CC1
13	LM26096		I	327	CC1	IVb	CC1
14	LM28619		I	63	CC1	IVb	CC1
15	LM34020		I	1	CC1	IVb	CC1
16	LM42629	42629	I	73	CC1	IVb	CC1
17	LM68828	68828	I	248	CC1	IVb	CC1
18	LM70927	70927	I	252	CC1	IVb	CC1
19	LM73014		I	134	CC1	IVb	CC1
20	LM77135	77135	I	278	CC1	IVb	CC1
21	LM84441	84441	I	385	CC1	IVb	CC1
22	LM85809		I	1	CC1	IVb	CC1
23	LM86256		I	62	CC1	IVb	CC1
24	SLCC1849		I	258	CC1	IVb	CC1
25	LM235		I	104	CC1	IVb	CC1
26	LM68		I	150	CC1	IVb	CC1
27	LM71		I	151	CC1	IVb	CC1
28	SLCC63		I	119	CC1	IVb	CC1
29	LM05-00105	CLIP2005/00105	I	272	CC2	IVb	CC2
30	LM06-01589	CLIP2006/01589	I	2	CC2	IVb	CC2
31	LM07-00231	CLIP2007/00231	I	2	CC2	IVb	CC2
32	LM07-01297	CLIP2007/01297	I	418	CC2	IVb	CC2

Table 10 (continued):

Study order	Identification result	Reference of MLST data	Origin	Year	Country
1	CC1	Ref. B	Food	1981	Canada
2	CC1	Ref. B	Food	1987	Switzerland
3	CC1	This study	Animal	Unknown	Switzerland
4	CC1	This study	Animal	Unknown	Switzerland
5	CC1	Ref. B	Human	1987	New Zealand
6	Other IVb	Ref. B	Unknown	1987	China
7	CC1	This study	Human	2013	France
8	CC1	Ref. B	Human	1989	France
9	CC1	Ref. B	Human	1990	France
10	CC1	Ref. B	Human	1992	France
11	CC1	Ref. B	Human	1992	France
12	CC1	Ref. B	Food	1992	France
13	CC1	Ref. B	Human	1992	France
14	CC1	Ref. B	Human	1992	France
15	CC1	Ref. B	Food	1992	France
16	CC1	Ref. B	Unknown	1993	United Kingdom
17	CC1	Ref. B	Human	1987	Sweden
18	CC1	Ref. B	Animal	< 1996	Greece
19	CC1	Ref. B	Unknown	Unknown	France
20	CC1	Ref. B	Human	Unknown	Chile
21	CC1	Ref. B	Environment	2000	France
22	CC1	Ref. B	Human	2000	France
23	Other IVb	Ref. B	Human	2000	France
24	CC1	Ref. B	Animal	1964	Switzerland
25	Other IVb	Ref. B	Human	1954	Canada
26	Other IVb	Ref. B	Human	1934	USA
27	Other IVb	Ref. B	Human	Unknown	USA, Kentucky
28	Other IVb	Ref. B	Human	1933	USA
29	CC2	Ref. B	Human	Unknown	Algeria
30	CC2	Ref. B	Food	Unknown	Colombia
31	CC2	Ref. B	Food	Unknown	Morocco
32	CC2	Ref. B	Human	2007	France

Table 10 (continued):

Study order	Strain code	Original name	Lineage	ST	Clonal complex or singleton	PCR serogroup	Target category
33	LM08-00502	CLIP2008/00502	I	411	CC2	IVb	CC2
34	LM09-00290	CLIP2009/00290	I	2	CC2	IVb	CC2
35	LM10-01417	Scott A, FSL J1-255	I	2	CC2	IVb	CC2
36	LM10-01444	TS38/L.3306, FSL J1-116	I	2	CC2	IVb	CC2
37	LM15843		I	50	CC2	IVb	CC2
38	LM18354	18354	I	244	CC2	IVb	CC2
39	LM42398		I	67	CC2	IVb	CC2
40	LM51415	51415	I	245	CC2	IVb	CC2
41	LM59597	59597	I	2	CC2	IVb	CC2
42	LM61388	61388	I	246	CC2	IVb	CC2
43	LM68802	68802	I	247	CC2	IVb	CC2
44	LM70320	70320	I	2	CC2	IVb	CC2
45	LM71962	71962	I	276	CC2	IVb	CC2
46	LM75327	75327	I	277	CC2	IVb	CC2
47	LM76305	76305	I	2	CC2	IVb	CC2
48	LM76641	76641	I	2	CC2	IVb	CC2
49	LM84220		I	51	CC2	IVb	CC2
50	LM88497		I	100	CC2	IVb	CC2
51	LM88513	88513	I	2	CC2	IVb	CC2
52	SLCC1531		I	2	CC2	IVb	CC2
53	SLCC299		I	2	CC2	IVb	CC2
54	SLCC527		I	291	CC2	IVb	CC2
55	SLCC537		I	257	CC2	IVb	CC2
56	SLCC796		I	259	CC2	IVb	CC2
57	LM19700	19700	I	145	CC2	IVb	CC2
58	LM05-00855	CLIP2005/00855	I	3	CC3	IIb	CC3
59	LM07-00306	CLIP2007/00306	I	3	CC3	IIb	CC3
60	LM07-00837	CLIP2007/00837	I	3	CC3	IIb	CC3
61	LM07-01095	CLIP2007/01095	I	3	CC3	IIb	CC3
62	LM09-01317	CLIP2009/01317	I	423	CC3	IIb	CC3
63	LM09-01411	CLIP2009/01411	I	3	CC3	IIb	CC3
64	LM10-01296	CLIP2010/01296	I	3	CC3	IIb	CC3

Table 10 (continued):

Study order	PCR assay IVb						PCR assay IIb				PCR assay IIallic					
	LMOf2365_2638	LMOf2365_1900	LMOf2365_2702	LMOATCC19117_2417	LMOL312_1102	LM1816_RS00050	LMOf2365_2638	Imo1077	M637_08170	LMOSLCC2482_0307	Imo0525	LM6179_0610	LMLG_RS02400	LMRG_02577	LM5578_1185	Imo1118
33	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0
34	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0
35	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0
36	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0
37	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0
38	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
39	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0
40	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0
41	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0
42	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0
43	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0
44	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0
45	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0
46	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0
47	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0
48	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0
49	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0
50	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0
51	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0
52	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0
53	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0
54	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0
55	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0
56	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0
57	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
58	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0
59	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0
60	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0
61	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0
62	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0
63	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0
64	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0

Table 10 (continued):

Study order	Identification result	Reference of MLST data	Origin	Year	Country
33	CC1	Ref. B	Human	2008	France
34	CC2	Ref. B	Human	2005	Tunisia
35	CC2	Ref. B	Human	1983	USA
36	CC2	Ref. B	Human	1988	United Kingdom
37	CC2	Ref. B	Human	1990	France
38	Other IVb	Ref. B	Human	1989	New Zealand
39	CC2	Ref. B	Human	1993	France
40	CC2	Ref. B	Human	1993	Switzerland
41	CC2	Ref. B	Human	1991	Spain
42	CC2	Ref. B	Food	1994	Italy
43	CC2	Ref. B	Human	1975	Sweden
44	CC2	Ref. B	Human	1995	New Zealand
45	CC2	Ref. B	Food	Unknown	Tunisia
46	CC2	Ref. B	Human	Unknown	Morocco
47	CC2	Ref. B	Human	1998	Algeria
48	CC2	Ref. B	Food	1998	Portugal
49	CC2	Ref. B	Human	2000	France
50	CC2	Ref. B	Environment	2000	France
51	CC2	Ref. B	Environment	1999	Mexico
52	CC2	Ref. B	Human	1962	Denmark
53	CC2	Ref. B	Human	1955	USA
54	CC2	Ref. B	Human	1957	USA
55	CC2	Ref. B	Human	Unknown	Canada
56	CC2	Ref. B	Animal	1959	Switzerland
57	Other IVb	Ref. B	Food	Unknown	Irak
58	CC3	Ref. B	Human	2005	France
59	CC3	Ref. B	Human	2007	France
60	CC3	Ref. B	Human	2007	France
61	CC3	Ref. B	Human	2007	France
62	CC3	Ref. B	Human	2009	France
63	CC3	This study	Human	2008	France
64	CC3	This study	Human	2009	France

Table 10 (continued):

Study order	Strain code	Original name	Lineage	ST	Clonal complex or singleton	PCR serogroup	Target category
65	LM10-01426	FSL J1-049	I	3	CC3	IIb	CC3
66	LM10-01453	G6003, FSL R2-502	I	3	CC3	IIb	CC3
67	LM11-00007	CLIP2011/00007	I	3	CC3	IIb	CC3
68	LM12-00337	CLIP2012/00337	I	3	CC3	IIb	CC3
69	LM13-00288	CLIP2013/00288	I	3	CC3	IIb	CC3
70	LM13419	13419	I	273	CC3	IIb	CC3
71	LM13903	13903	I	225	CC3	IIb	CC3
72	LM15262	15262	I	228	CC3	IIb	CC3
73	LM16314		I	39	CC3	IIb	CC3
74	LM18327	18327	I	41	CC3	IIb	CC3
75	LM19695	19695	I	66	CC3	IIb	CC3
76	LM19866		I	42	CC3	IIb	CC3
77	LM74917		I	44	CC3	IIb	CC3
78	LM77141	77141	I	280	CC3	IIb	CC3
79	LM77152	77152	I	281	CC3	IIb	CC3
80	LM77479	77479	I	68	CC3	IIb	CC3
81	LM80112	80112	I	235	CC3	IIb	CC3
82	LM80547	80547	I	117	CC3	IIb	CC3
83	LM80914		I	3	CC3	IIb	CC3
84	LM83775		I	3	CC3	IIb	CC3
85	LM86505	86505	I	287	CC3	IIb	CC3
86	LM05-00646	CLIP2005/00646	I	4	CC4	IVb	CC4
87	LM07-01318	CLIP2007/01318	I	4	CC4	IVb	CC4
88	LM08-00123	CLIP2010/00128	I	408	CC4	IVb	CC4
89	LM08-01352	CLIP2008/01352	I	4	CC4	IVb	CC4
90	LM09-00028	CLIP2009/00028	I	4	CC4	IVb	CC4
91	LM09-00558	CLIP2009/00558	I	4	CC4	IVb	CC4
92	LM09-00659	CLIP2009/00659	I	4	CC4	IVb	CC4
93	LM09-00994	CLIP2009/00994	I	4	CC4	IVb	CC4
94	LM10-00740	CLIP2010/00740	I	4	CC4	IVb	CC4
95	LM11-00181	CLIP2011/00181	I	4	CC4	IVb	CC4
96	LM11-00946	CLIP2011/00946	I	4	CC4	IVb	CC4

Table 10 (continued):

Study order	Identification result	Reference of MLST data	Origin	Year	Country
65	CC3	Ref. B	Human	2010	Unknown
66	CC3	Ref. B	Food	1994	USA
67	CC3	This study	Human	2009	France
68	CC3	This study	Human	2009	France
69	CC3	This study	Human	2010	France
70	CC3	Ref. B	Human	1987	Algeria
71	CC3	Ref. B	Food	1989	Turkey
72	CC3	Ref. B	Human	1990	France
73	CC3	Ref. B	Human	1990	France
74	CC3	Ref. B	Human	1990	Morocco
75	CC3	Ref. B	Food	Unknown	Irak
76	CC3	Ref. B	Human	1991	France
77	CC3	Ref. B	Unknown	Unknown	France
78	CC3	Ref. B	Food	Unknown	Chile
79	CC3	Ref. B	Food	Unknown	Chile
80	CC3	Ref. B	Vegetable	Unknown	Morocco
81	CC3	Ref. B	Animal	< 2000	Croatia
82	CC3	Ref. B	Food	1999	Argentina
83	CC3	Ref. B	Human	2000	France
84	CC3	Ref. B	Human	2000	France
85	CC3	Ref. B	Food	Unknown	Morocco
86	CC4	Ref. B	Human	2005	Unknown
87	CC4	Ref. B	Human	2007	Unknown
88	CC4	This study	Human	2010	France
89	CC4	Ref. B	Human	2008	France
90	CC4	Ref. B	Human	2009	France
91	CC4	Ref. B	Human	2009	France
92	CC4	Ref. B	Human	2009	France
93	CC4	Ref. B	Human	2009	France
94	CC4	This study	Human	2010	France
95	CC4	This study	Human	2010	France
96	CC4	This study	Human	2010	France

Table 10 (continued):

Study order	Strain code	Original name	Lineage	ST	Clonal complex or singleton	PCR serogroup	Target category
97	LM12-00190	CLIP2012/00190	I	4	CC4	IVb	CC4
98	LM13-00207	CLIP2013/00207	I	4	CC4	IVb	CC4
99	LM13630		I	55	CC4	IVb	CC4
100	LM21131	21131	I	4	CC4	IVb	CC4
101	LM32		I	146	CC4	IVb	CC4
102	LM64		I	118	CC4	IVb	CC4
103	LM70290	70290	I	251	CC4	IVb	CC4
104	LM82		I	125	CC4	IVb	CC4
105	LM84345		I	4	CC4	IVb	CC4
106	LM05-00704	CLIP2005/00704	I	5	CC5	IIb	CC5
107	LM08-00225	CLIP2008/00225	I	409	CC5	IIb	CC5
108	LM09-00364	CLIP2009/00364	I	5	CC5	IIb	CC5
109	LM09-00390	CLIP2009/00390	I	5	CC5	IIb	CC5
110	LM09-01328	CLIP2009/01328	I	5	CC5	IIb	CC5
111	LM10-00003	CLIP2010/00003	I	5	CC5	IIb	CC5
112	LM10-00192	CLIP2010/00192	I	5	CC5	IIb	CC5
113	LM10-01335	CLIP2010/01335	I	5	CC5	IIb	CC5
114	LM10-01422	FSL J2-064	I	5	CC5	IIb	CC5
115	LM10-01425	FSL J1-169	I	5	CC5	IIb	CC5
116	LM11-00140	CLIP2011/00140	I	5	CC5	IIb	CC5
117	LM11-00317	CLIP2011/00317	I	5	CC5	IIb	CC5
118	LM11-00927	CLIP2011/00927	I	5	CC5	IIb	CC5
119	LM11-00992	CLIP2011/00992	I	5	CC5	IIb	CC5
120	LM12-00187	CLIP2012/00187	I	5	CC5	IIb	CC5
121	LM12-00706	CLIP2012/00706	I	5	CC5	IIb	CC5
122	LM12-01096	CLIP2012/01096	I	5	CC5	IIb	CC5
123	LM80731		I	5	CC5	IIb	CC5
124	LM82900	82900	I	5	CC5	IIb	CC5
125	LM86102		I	5	CC5	IIb	CC5
126	LM95742	2004/95742	I	5	CC5	IIb	CC5
127	LM07-00576	CLIP2007/00576	I	443	CC6	IVb	CC6
128	LM07-01127	CLIP2007/01127	I	6	CC6	IVb	CC6

Table 10 (continued):

Study order	Identification result	Reference of MLST data	Origin	Year	Country
97	CC4	This study	Human	2010	France
98	CC4	This study	Human	2010	France
99	CC4	Ref. B	Human	1989	France
100	CC4	Ref. B	Food	1992	France
101	CC4	Ref. B	Human	Unknown	Unknown
102	CC4	Ref. B	Human	1934	USA
103	CC4	Ref. B	Human	< 1996	Greece
104	CC4	Ref. B	Unknown	Unknown	Unknown
105	CC4	Ref. B	Human	2000	France
106	CC5	Ref. B	Human	2005	France
107	CC5	Ref. B	Human	2008	France
108	CC5	Ref. B	Human	2009	France
109	CC5	Ref. B	Human	2009	France
110	CC5	This study	Human	2010	France
111	CC5	This study	Human	2010	France
112	CC5	Ref. B	Human	2010	France
113	CC5	This study	Human	2010	France
114	CC5	Ref. B	Animal	1989	Unknown
115	CC5	Ref. B	Human	1996	Unknown
116	CC5	This study	Human	2010	France
117	CC5	This study	Human	2010	France
118	CC5	This study	Human	2010	France
119	CC5	This study	Human	2010	France
120	CC5	This study	Human	2010	France
121	CC5	This study	Human	2010	France
122	CC5	This study	Human	2010	France
123	CC5	Ref. B	Human	2000	France
124	CC5	Ref. A	Animal	< 2000	Germany
125	CC5	Ref. B	Human	2000	France
126	CC5	Ref. B	Human	2004	France
127	CC6	Ref. B	Food	2007	Unknown
128	CC6	Ref. B	Food	< 2007	Netherlands

Table 10 (continued):

Study order	Strain code	Original name	Lineage	ST	Clonal complex or singleton	PCR serogroup	Target category
129	LM09-01048	CLIP2009/01048	I	442	CC6	IVb	CC6
130	LM10-00870	CLIP2010/00870	I	6	CC6	IVb	CC6
131	LM10-01084	CLIP2010/01084	I	6	CC6	IVb	CC6
132	LM10-01418	H7550, FSL N1-225	I	6	CC6	IVb	CC6
133	LM10-01455	J1735, FSL R2-763	I	6	CC6	IVb	CC6
134	LM10-01456	J1925, FSL R2-764	I	6	CC6	IVb	CC6
135	LM10-01520	CLIP2010/01520	I	6	CC6	IVb	CC6
136	LM11-01044	CLIP2011/01044	I	6	CC6	IVb	CC6
137	LM12-00171	CLIP2012/00171	I	6	CC6	IVb	CC6
138	LM12-01046	CLIP2012/01046	I	6	CC6	IVb	CC6
139	LM12-01687	CLIP2012/01687	I	6	CC6	IVb	CC6
140	LM13-00103	CLIP2013/00103	I	6	CC6	IVb	CC6
141	LM67774	67774	I	6	CC6	IVb	CC6
142	LM67783	67783	I	6	CC6	IVb	CC6
143	LM81438		I	6	CC6	IVb	CC6
144	LM88501	88501	I	6	CC6	IVb	CC6
145	LM94402		I	6	CC6	IVb	CC6
146	LM08-00730	CLIP2008/00730	II	7	CC7	IIa	CC7
147	LM10-00736	CLIP2010/00736	II	624	CC7	IIa	CC7
148	LM10-01031	CLIP2010/01031	II	7	CC7	IIa	CC7
149	LM10-01427	FSL C1-056	II	7	CC7	IIa	CC7
150	LM11-00951	CLIP2011/00951	II	7	CC7	IIa	CC7
151	LM12-00115	CLIP2012/00115	II	691	CC7	IIa	CC7
152	LM12-00390	CLIP2012/00390	II	7	CC7	IIa	CC7
153	LM12-01275	CLIP2012/01275	II	7	CC7	IIa	CC7
154	LM12-01805	CLIP2012/01805	II	7	CC7	IIa	CC7
155	LM24902	24902	II	7	CC7	IIa	CC7
156	LM305		II	105	CC7	IIa	CC7
157	LM307		II	107	CC7	IIa	CC7
158	LM36		II	111	CC7	IIa	CC7
159	LM502		II	116	CC7	IIa	CC7
160	LM56346	56346	II	7	CC7	IIa	CC7

Table 10 (continued):

Study order	PCR assay IVb						PCR assay IIb				PCR assay IIal1c					
	LMOf2365_2638	LMOf2365_1900	LMOf2365_2702	LMOATCC19117_2417	LMOL312_1102	LM1816_RS00050	LMOf2365_2638	Imo1077	M637_08170	LMOSLCC2482_0307	Imo0525	LM6179_0610	LM1G_RS02400	LMRG_02577	LM5578_1185	Imo1118
129	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0
130	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0
131	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0
132	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0
133	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0
134	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0
135	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0
136	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0
137	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0
138	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0
139	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0
140	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0
141	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0
142	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0
143	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0
144	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0
145	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0
146	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0
147	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0
148	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0
149	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0
150	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0
151	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0
152	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0
153	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0
154	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0
155	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0
156	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0
157	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0
158	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0
159	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0
160	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0

Table 10 (continued):

Study order	Identification result	Reference of MLST data	Origin	Year	Country
129	CC1	Ref. B	Human	2009	France
130	CC6	This study	Human	2010	France
131	CC6	This study	Human	2010	France
132	CC6	Ref. B	Human	1998	USA
133	CC6	Ref. B	Human	2002	USA
134	CC6	Ref. B	Food	2002	USA
135	CC6	This study	Human	2010	France
136	CC6	This study	Human	2010	France
137	CC6	This study	Human	2010	France
138	CC6	This study	Human	2010	France
139	CC6	This study	Human	2010	France
140	CC6	This study	Human	2010	France
141	CC6	Ref. B	Environment	< 1995	Greece
142	CC6	Ref. B	Food	< 1995	Greece
143	CC6	Ref. B	Human	2000	France
144	CC6	Ref. B	Environment	1999	Mexico
145	CC6	Ref. B	Human	2003	France
146	CC7	Ref. B	Human	2008	France
147	CC7	This study	Human	2011	France
148	CC7	This study	Human	2011	France
149	CC7	Ref. B	Human	1998	Unknown
150	CC7	This study	Human	2011	France
151	CC7	This study	Human	2011	France
152	CC7	This study	Human	2011	France
153	CC7	This study	Human	2011	France
154	CC7	This study	Human	2011	France
155	CC7	Ref. B	Food	1992	United Kingdom
156	CC7	Ref. B	Human	1952	Unknown
157	CC7	Ref. B	Human	1953	Germany
158	CC7	Ref. B	Human	1953	Germany
159	CC7	Ref. B	Animal	Unknown	France
160	CC7	Ref. B	Animal	1993	Israel

Table 10 (continued):

Study order	Strain code	Original name	Lineage	ST	Clonal complex or singleton	PCR serogroup	Target category
161	LM62444	62444	II	231	CC7	Ila	CC7
162	LM71350	71350	II	12	CC7	Ila	CC7
163	LM8077	8077	II	7	CC7	Ila	CC7
164	LM86255		II	7	CC7	Ila	CC7
165	LM87		II	158	CC7	Ila	CC7
166	LM8824		II	24	CC7	Ila	CC7
167	SLCC1		II	23	CC7	Ila	CC7
168	SLCC2864		II	7	CC7	Ila	CC7
169	LM05-00191	CLIP2005/00191	II	8	CC8	Ila	CC8
170	LM06-00694	CLIP2006/00694	II	8	CC8	Ila	CC8
171	LM06-01462	CLIP2006/01462	II	8	CC8	Ila	CC8
172	LM07-00511	CLIP2007/00511	II	8	CC8	Ila	CC8
173	LM07-01074	CLIP2007/01074	II	8	CC8	Ila	CC8
174	LM07-01393	CLIP2007/01393	II	8	CC8	Ila	CC8
175	LM09-00871	CLIP2009/00871	II	8	CC8	Ila	CC8
176	LM10-00336	CLIP2010/00336	II	8	CC8	Ila	CC8
177	LM11-00658	CLIP2011/00658	II	8	CC8	Ila	CC8
178	LM11-00987	CLIP2011/00987	II	8	CC8	Ila	CC8
179	LM12-01023	CLIP2012/01023	II	8	CC8	Ila	CC8
180	LM12-01049	CLIP2012/01049	II	8	CC8	Ila	CC8
181	LM13-00064	CLIP2013/00064	II	8	CC8	Ila	CC8
182	LM13-00094	CLIP2013/00094	II	8	CC8	Ila	CC8
183	LM62719	62719	II	232	CC8	Ila	CC8
184	LM68619	68619	II	120	CC8	Ila	CC8
185	LM68625	68625	II	8	CC8	Ila	CC8
186	LM90291	90291	II	289	CC8	Ila	CC8
187	LM12-01748	CLIP2012/01748	II	9	CC9	Ilc	CC9
188	EGDe-CNR	EGDe-CNR	II	35	CC9	Ilc	CC9
189	LM05-01099	CLIP2005/01099	II	9	CC9	Ilc	CC9
190	LM06-00139	CLIP2006/00139	II	9	CC9	Ilc	CC9
191	LM06-00810	CLIP2006/00810	II	9	CC9	Ilc	CC9
192	LM07-01124	CLIP2007/01124	II	223	CC9	Ilc	CC9

Table 10 (continued):

Study order	PCR assay IVb						PCR assay IIb				PCR assay IIal1c					
	LMOf2365_2638	LMOf2365_1900	LMOf2365_2702	LMOATCC19117_2417	LMOL312_1102	LM1816_RS00050	LMOf2365_2638	Imo1077	M637_08170	LMOSLCC2482_0307	Imo0525	LM6179_0610	LM1G_RS02400	LMRG_02577	LM5578_1185	Imo1118
161	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0
162	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0
163	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0
164	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0
165	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0
166	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0
167	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0
168	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0
169	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0
170	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0
171	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0
172	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0
173	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0
174	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0
175	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0
176	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0
177	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0
178	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0
179	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0
180	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0
181	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0
182	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0
183	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0
184	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0
185	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0
186	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0
187	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
188	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1
189	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1
190	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1
191	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1
192	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1

Table 10 (continued):

Study order	Identification result	Reference of MLST data	Origin	Year	Country
161	CC7	Ref. B	Food	1995	France
162	CC7	Ref. B	Food	Unknown	Iran
163	CC7	Ref. B	Food	1987	Finland
164	CC7	Ref. B	Human	2000	France
165	CC7	Ref. B	Animal	Unknown	United Kingdom
166	CC7	Ref. B	Human	1988	France
167	CC7	Ref. B	Human	1951	Germany
168	CC7	Ref. B	Food	1968	Germany
169	CC8	Ref. B	Human	2005	France
170	CC8	Ref. B	Human	2006	France
171	CC8	Ref. B	Human	2006	France
172	CC8	Ref. B	Human	2007	France
173	CC8	Ref. B	Human	2007	France
174	CC8	Ref. B	Human	2007	France
175	CC8	Ref. B	Human	2009	France
176	CC8	Ref. B	Human	2010	France
177	CC8	This study	Human	2011	France
178	CC8	This study	Human	2011	France
179	CC8	This study	Human	2011	France
180	CC8	This study	Human	2011	France
181	CC8	This study	Human	2011	France
182	CC8	This study	Human	2011	France
183	CC8	Ref. B	Human	1994	New Zealand
184	CC8	Ref. B	Human	1995	Italy
185	CC8	Ref. B	Human	1992	Algeria
186	CC8	Ref. B	Food	Unknown	Chile
187	Other lineage II	This study	Human	2011	France
188	CC9	Ref. B	Unknown	1960	Europe
189	CC9	Ref. B	Human	< 2005	Portugal
190	CC9	Ref. B	Environment	2006	France
191	CC9	Ref. B	Environment	2005	Italy
192	CC9	Ref. B	Human	< 2007	Netherlands

Table 10 (continued):

Study order	Strain code	Original name	Lineage	ST	Clonal complex or singleton	PCR serogroup	Target category
193	LM07-01172	CLIP2007/01172	II	9	CC9	IIc	CC9
194	LM10-01314	CLIP2010/01314	II	9	CC9	IIc	CC9
195	LM11-00056	CLIP2011/00056	II	9	CC9	IIc	CC9
196	LM11308	11308	II	9	CC9	IIc	CC9
197	LM12-00378	CLIP2012/00378	II	9	CC9	IIc	CC9
198	LM12485	12485	II	9	CC9	IIc	CC9
199	LM14228	14228	II	9	CC9	IIc	CC9
200	LM15183	15183	II	9	CC9	IIc	CC9
201	LM317		II	34	CC9	IIc	CC9
202	LM327		II	110	CC9	IIc	CC9
203	LM67781	67781	II	115	CC9	IIc	CC9
204	LM70956	70956	II	9	CC9	IIc	CC9
205	LM71634	71634	II	9	CC9	IIc	CC9
206	LM762		II	123	CC9	IIc	CC9
207	LM8742	8742	II	9	CC9	IIc	CC9
208	LM87494		II	138	CC9	IIc	CC9
209	LM8796	8796	II	9	CC9	IIc	CC9
210	LM9134	9134	II	9	CC9	IIc	CC9
211	SLCC21		II	122	CC9	IIc	CC9
212	LM07-00581	CLIP2007/00581	II	121	CC121	IIa	CC121
213	LM07-01014	CLIP2007/01014	II	121	CC121	IIa	CC121
214	LM08-01095	CLIP2008/01095	II	121	CC121	IIa	CC121
215	LM10-00211	CLIP2010/00211	II	121	CC121	IIa	CC121
216	LM10-01259	CLIP2010/01259	II	121	CC121	IIa	CC121
217	LM11-00013	CLIP2011/00013	II	121	CC121	IIa	CC121
218	LM12-00189	CLIP2012/00189	II	121	CC121	IIa	CC121
219	LM12-00200	CLIP2012/00200	II	121	CC121	IIa	CC121
220	LM13394	13394	II	121	CC121	IIa	CC121
221	LM15178	15178	II	121	CC121	IIa	CC121
222	LM22261	22261	II	121	CC121	IIa	CC121
223	LM310		II	108	CC121	IIa	CC121
224	LM70124	70124	II	121	CC121	IIa	CC121

Table 10 (continued):

Study order	PCR assay IVb						PCR assay IIb				PCR assay IIal1c					
	LMOf2365_2638	LMOf2365_1900	LMOf2365_2702	LMOATCC19117_2417	LMOL312_1102	LM1816_RS00050	LMOf2365_2638	Imo1077	M637_08170	LMOSLCC2482_0307	Imo0525	LM6179_0610	LMLG_RS02400	LMRG_02577	LM5578_1185	Imo1118
193	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1
194	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1
195	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1
196	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1
197	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1
198	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1
199	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1
200	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1
201	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1
202	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1
203	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1
204	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1
205	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1
206	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1
207	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1
208	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1
209	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1
210	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1
211	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1
212	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0
213	1	1	0	0	0	0	0	1	0	0	1	1	0	0	0	0
214	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0
215	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0
216	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0
217	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0
218	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0
219	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0
220	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0
221	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0
222	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0
223	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0
224	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0

Table 10 (continued):

Study order	Identification result	Reference of MLST data	Origin	Year	Country
193	CC9	Ref. B	Animal	2003	Italy
194	CC9	This study	Human	2011	France
195	CC9	This study	Human	2011	France
196	CC9	Ref. B	Human	1988	New Zealand
197	CC9	This study	Human	2011	France
198	CC9	Ref. B	Food	1988	France
199	CC9	Ref. B	Food	1989	Denmark
200	CC9	Ref. B	Human	1989	Finland
201	CC9	Ref. B	Human	1955	Germany
202	CC9	Ref. B	Unknown	1955	USA, Chicago
203	CC9	Ref. B	Food	< 1995	Greece
204	CC9	Ref. B	Animal	< 1996	Greece
205	CC9	Ref. B	Food	< 1996	Portugal
206	CC9	Ref. B	Animal	1941	Unknown
207	CC9	Ref. B	Food	1986	Austria
208	CC9	Ref. B	Environment	2001	France
209	CC9	Ref. B	Food	1988	Austria
210	CC9	Ref. B	Food	1988	Spain
211	CC9	Ref. B	Human	1935	United Kingdom
212	CC 121	Ref. A	Food	1990	Belgium
213	CC 121	Ref. B	Food	2007	Unknown
214	CC 121	Ref. A	Human	< 2005	Portugal
215	CC 121	This study	Human	2011	France
216	CC 121	This study	Human	2011	France
217	CC 121	This study	Human	2011	France
218	CC 121	This study	Human	2011	France
219	CC 121	This study	Human	2011	France
220	CC 121	Ref. A	Food	1989	Italy
221	CC 121	Ref. A	Human	1989	Finland
222	CC 121	Ref. A	Food	1992	China
223	CC 121	Ref. B	Human	1954	Germany
224	CC 121	Ref. A	Environment	< 1996	Portugal

Table 10 (continued):

Study order	Strain code	Original name	Lineage	ST	Clonal complex or singleton	PCR serogroup	Target category
225	LM82806	82806	II	121	CC121	Ila	CC121
226	LM10-01410	CLIP2010/01410	II	155	CC155	Ila	CC155
227	LM11-00399	CLIP2011/00399	II	155	CC155	Ila	CC155
228	LM11-00429	CLIP2011/00429	II	155	CC155	Ila	CC155
229	LM11-00875	CLIP2011/00875	II	155	CC155	Ila	CC155
230	LM12-00006	CLIP2012/00006	II	155	CC155	Ila	CC155
231	LM18427	18427	II	155	CC155	Ila	CC155
232	LM21413	21413	II	155	CC155	Ila	CC155
233	LM10-01447	TS4/F.6854, FSL N3-031	II	11	CC11	Ila	Other lineage II
234	LM10-00128	CLIP2010/00128	II	206	CC14	Ila	Other lineage II
235	LM12-00549	CLIP2012/00549	II	399	CC14	Ila	Other lineage II
236	LM12-00705	CLIP2012/00705	II	14	CC14	Ila	Other lineage II
237	LM13-00005	CLIP2013/00005	II	91	CC14	Ila	Other lineage II
238	LM13-00282	CLIP2013/00282	II	428	CC177	Ila	Other lineage II
239	LM84803		II	18	CC18	Ila	Other lineage II
240	LM83088	83088	II	19	CC19	Ila	Other lineage II
241	LM12-01094	CLIP2012/01094	II	199	CC199	Ila	Other lineage II
242	LM10-00223	CLIP2010/00223	II	20	CC20	Ila	Other lineage II
243	LM11-00863	CLIP2011/00863	II	424	CC20	Ila	Other lineage II
244	LM10-00509	CLIP2010/00509	II	204	CC204	Ila	Other lineage II
245	LM12-00009	CLIP2012/00009	II	694	CC204	Ila	Other lineage II
246	LM10-01106	CLIP2010/01106	II	695	CC207	Ila	Other lineage II
247	LM11-00942	CLIP2011/00942	II	207	CC207	Ila	Other lineage II
248	LM10-00210	CLIP2010/00210	II	21	CC21	Ila	Other lineage II
249	LM12-00849	CLIP2012/00849	II	697	CC21	Ila	Other lineage II
250	LM11-00331	CLIP2011/00331	II	29	CC29	Ila	Other lineage II
251	LM11-00624	CLIP2011/00624	II	31	CC31	Ila	Other lineage II
252	LM10-00408	CLIP2010/00408	II	37	CC37	Ila	Other lineage II
253	LM10-01430	FSL J2-031	II	394	CC394	Ila	Other lineage II
254	LM10-01428	CU-BR1/93, FSL J2-054	II	412	CC412	Ila	Other lineage II
255	LM10-01432	FSL J2-063	II	16	CC16	Ila	Other lineage II
256	LM11-00067	CLIP2011/00067	II	124	singleton ST124	Ila	Other lineage II

Table 10 (continued):

Study order	PCR assay IVb						PCR assay IIb				PCR assay IIal1c					
	LMOf2365_2638	LMOf2365_1900	LMOf2365_2702	LMOATCC19117_2417	LMOL312_1102	LM1816_RS00050	LMOf2365_2638	Imo1077	M637_08170	LMOSLCC2482_0307	Imo0525	LM6179_0610	LMLG_RS02400	LMRG_02577	LM5578_1185	Imo1118
225	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0
226	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0
227	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0
228	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0
229	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0
230	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0
231	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0
232	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0
233	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
234	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
235	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
236	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
237	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
238	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
239	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
240	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
241	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
242	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
243	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
244	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
245	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
246	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
247	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
248	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
249	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
250	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
251	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
252	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
253	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
254	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
255	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0
256	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0

Table 10 (continued):

Study order	Identification result	Reference of MLST data	Origin	Year	Country
225	CC 121	Ref. A	Food	< 2000	Germany
226	CC 155	This study	Human	2011	France
227	CC 155	This study	Human	2011	France
228	CC 155	This study	Human	2011	France
229	CC 155	This study	Human	2011	France
230	CC 155	This study	Human	2011	France
231	CC 155	Ref. A	Human	1991	New Zealand
232	CC 155	Ref. A	Human	1991	China
233	Other lineage II	Ref. B	Food	1989	USA
234	Other lineage II	This study	Human	2011	France
235	Other lineage II	This study	Human	2012	France
236	Other lineage II	This study	Human	2012	France
237	Other lineage II	This study	Human	2012	France
238	Other lineage II	This study	Human	2012	France
239	Other lineage II	Ref. B	Human	2000	France
240	Other lineage II	Ref. B	Human	2000	France
241	Other lineage II	This study	Human	2012	France
242	Other lineage II	This study	Human	2012	France
243	Other lineage II	This study	Human	2012	France
244	Other lineage II	This study	Human	2012	France
245	Other lineage II	This study	Human	2012	France
246	Other lineage II	This study	Human	2012	France
247	Other lineage II	This study	Human	2012	France
248	Other lineage II	This study	Human	2012	France
249	Other lineage II	This study	Human	2012	France
250	Other lineage II	This study	Human	2012	France
251	Other lineage II	This study	Human	2012	France
252	Other lineage II	This study	Human	2012	France
253	Other lineage II	Ref. B	Animal	1996	Unknown
254	Other lineage II	Ref. B	Animal	1993	Unknown
255	CC8	Ref. B	Animal	1993	Unknown
256	Other lineage II	This study	Human	2012	France

Table 10 (continued):

Study order	Strain code	Original name	Lineage	ST	Clonal complex or singleton	PCR serogroup	Target category
257	LM11-00421	CLIP2011/00421	II	36	singleton ST36	IIa	Other lineage II
258	LM10-01431	FSL J2-066	II	376	singleton ST376	IIa	Other lineage II
259	LM09-01364	CLIP2009/01364	II	398	singleton ST398	IIa	Other lineage II
260	LM10-00519	CLIP2010/00519	II	403	singleton ST403	IIa	Other lineage II
261	LM10-00868	CLIP2010/00868	II	451	singleton ST451	IIa	Other lineage II
262	LM11-00015	CLIP2011/00015	II	504	singleton ST504	IIa	Other lineage II
263	LM10-01419	FSL J2-020	II	556	singleton ST556	IIa	Other lineage II
264	LM11-00371	CLIP2011/00371	II	200	singleton ST200	IIa	Other lineage II
265	LM10-01423	FSL J1-177	I	288	CC288	IIb	Other IIb
266	LM10-01424	CU-BR9/93, FSL J2-035	I	59	CC59	IIb	Other IIb
267	LM10-01083	CLIP2010/01083	I	77	CC77	IIb	Other IIb
268	LM12-01364	CLIP2012/01364	I	687	CC77	IIb	Other IIb
269	LM12-01497	CLIP2012/01497	I	686	CC77	IIb	Other IIb
270	LM11-00217	CLIP2011/00217	I	560	singleton ST560	IIb	Other IIb
271	LM11-00045	CLIP2011/00045	I	87	singleton ST87	IIb	Other IIb
272	LM12-00555	CLIP2012/00555	I	696	CC220	IVb	Other IVb
273	LM11-01208	CLIP2011/01208	I	194	CC315	IVb	Other IVb
274	LM10-01572	CLIP2010/01572	I	218	singleton ST218	IVb	Other IVb
275	LM10-00950	CLIP2010/00950	I	219	singleton ST219	IVb	Other IVb
276	LM13-00209	CLIP2013/00209	I	693	singleton ST240	IVb	Other IVb
277	LM84790	84790	I	255	singleton ST255	IVb	Other IVb
278	LM12-00070	CLIP2012/00070	I	382	singleton ST382	IVb	Other IVb
279	LM11-00198	CLIP2011/00198	I	388	singleton ST388	IVb	Other IVb
280	LM12-00648	CLIP2012/00648	I	389	singleton ST389	IVb	Other IVb
281	LM10-00241	CLIP2010/00241	I	54	singleton ST54	IVb	Other IVb
282	LM10-01451	J0144, FSL R2-500	I	558	singleton ST558	IVb	Other IVb

Table 10 (continued):

Study order	PCR assay IVb						PCR assay IIb				PCR assay IIal1c					
	<i>LMO</i> f2365_2638	<i>LMO</i> f2365_1900	<i>LMO</i> f2365_2702	<i>LMO</i> ATCC19117_2417	<i>LMOL</i> 312_1102	<i>LM</i> 1816_RS00050	<i>LMO</i> f2365_2638	<i>lmo</i> 1077	<i>M</i> 637_08170	<i>LMOSLCC</i> 2482_0307	<i>lmo</i> 0525	<i>LM</i> 6179_0610	<i>LM</i> LG_RS02400	<i>LM</i> RG_02577	<i>LM</i> 5578_1185	<i>lmo</i> 1118
257	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
258	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
259	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
260	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
261	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
262	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
263	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
264	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
265	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
266	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
267	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
268	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
269	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
270	1	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0
271	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
272	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
273	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
274	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0
275	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
276	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
277	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0
278	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
279	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
280	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
281	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
282	1	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0

Table 10 (continued and end):

Study order	Identification result	Reference of MLST data	Origin	Year	Country
257	Other lineage II	This study	Human	2012	France
258	Other lineage II	Ref. B	Animal	1994	Unknown
259	Other lineage II	This study	Human	2012	France
260	Other lineage II	This study	Human	2012	France
261	Other lineage II	This study	Human	2012	France
262	Other lineage II	This study	Human	2012	France
263	Other lineage II	Ref. B	Animal	1986	Unknown
264	Other lineage II	This study	Human	2013	France
265	Other IIb	Ref. B	Human	1997	Unknown
266	Other IIb	Ref. B	Animal	1993	Unknown
267	Other IIb	This study	Human	2012	France
268	Other IIb	This study	Human	2012	France
269	Other IIb	This study	Human	2012	France
270	Other IIb	This study	Human	2012	France
271	Other IIb	This study	Human	2012	France
272	Other IVb	This study	Human	2012	France
273	Other IVb	This study	Human	2012	France
274	CC1	This study	Human	2013	France
275	Other IVb	This study	Human	2013	France
276	Other IVb	This study	Human	2013	France
277	CC1	Ref. B	Environment	< 2000	Portugal
278	Other IVb	This study	Human	2013	France
279	Other IVb	This study	Human	2013	France
280	Other IVb	This study	Human	2013	France
281	Other IVb	This study	Human	2013	France
282	Other IVb	Ref. B	Food	2000	USA

Ref A : Chenal-Francisque et al., 2011

5 Ref B : Chenal-Francisque et al., 2013

Table 11. Identification results.

CC	No. isolates	No. of correctly identified strains (%)	No. of incomplete identification (%)	No. of incorrect identification (%)
CC1	28	22 (78.6)	6 strains identified as other IVb (21.4%)	
CC2	29	27 (93.1)	2 strains identified as other IVb (6.9%)	
CC3	28	28 (100)		
CC4	20	20 (100)		
CC5	21	21 (100)		
CC6	19	18 (94.7)		1 strain identified as CC1 (5.3%)
CC7	23	23 (100)		
CC8	18	18 (100)		
CC9	25	24 (96)	1 strain identified as other lineage II (4.0%)	
CC121	14	14 (100)		
CC155	7	7 (100)		
Other IVb	11	9 (81.8)		2 strains identified as CC1 (18.2%)
Other IIb	7	7 (86)		
Other lineage II	32	31 (96.9)		1 strain identified as CC8 (3.1%)
Total	282	269 (95.4%)	9 (3.2%)	4 (1.4%)

BIBLIOGRAPHIC REFERENCES

- Brosch**, Chen and Luchansky 1994. Pulsed-field fingerprinting of *Listeriae*: identification of genomic divisions for *Listeria monocytogenes* and their correlation with serovar. *Appl. Environ. Microbiol.* 60: 2584–2592.
- 5 **Cantinelli** T, Chenal-Francisque V, Diancourt L, Frezal L, Leclercq A, Wirth T, Lecuit M, Brisse S. 2013. "Epidemic clones" of *Listeria monocytogenes* are widespread and ancient clonal groups. *J Clin Microbiol* 51:3770-3779.
- Chenal-Francisque** V, Lopez J, Cantinelli T, Caro V, Tran C, Leclercq A, Lecuit M, Brisse S. 2011. Worldwide distribution of major clones of *Listeria monocytogenes*. *Emerg Infect Dis* 17:1110-1112.
- 10 **Chenal-Francisque** V, Diancourt L, Cantinelli T, Passet V, Tran-Hykes C, Bracq-Dieye H, Leclercq A, Pourcel C, Lecuit M, Brisse S. 2013. An optimized MLVA assay and its complementarity with PFGE and MLST for *Listeria monocytogenes* clone identification and surveillance. *J Clin Microbiol* 61.
- 15 **Doumith**, Buchrieser, Glaser, Jacquet and Martin 2004. Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. *J. Clin. Microbiol.* 42:3819-3822.
- Graves** LM, Swaminathan B. PulseNet standardized protocol for subtyping *Listeria monocytogenes* by macrorestriction and pulsed-field gel electrophoresis. *Int J Food Microbiol* 2001 Apr 11; 65(1-2):55-62.
- 20 **Haase** JK, Didelot X, Lecuit M, Korkeala H, Group LmMS, Achtman M. 2014. The ubiquitous nature of *Listeria monocytogenes* clones: a large-scale Multilocus Sequence Typing study. *Environ Microbiol* 16:405-416.
- Ragon**, Wirth, Hollandt, Lavenir, Lecuit, Le Monnier and Brisse 2008. A new perspective on *Listeria monocytogenes* evolution. *PLoS Pathog.* 4:e1000146.
- 25 **Seeliger** and Hohne 1979. Serotyping of *Listeria monocytogenes* and related species. *Methods Microbiol.*: 31–49.
- Ward**, Ducey, Usgaard, Dunn, Bielawski 2008. Multilocus genotyping assays for single nucleotide polymorphism-based subtyping of *Listeria monocytogenes* isolates. *Appl. Environ. Microbiol.*, 74 (2008), pp. 7629–7642.

CLAIMS

1. An *in vitro* method for determining that an isolate of *Listeria monocytogenes* has a high probability of belonging to clone CC1, to clone CC2, to clone CC4, to clone CC6, to clone CC7, to clone CC3, to clone CC5, to clone CC8 or CC16, or to clone CC155 of *L. monocytogenes*, wherein a high probability of belonging to one of these clones is indicative that said *L. monocytogenes* isolate has a potentiality, more particularly a high potentiality, of causing invasive infection in a human, wherein said method comprises detecting that one of the following genes i.-ix. is present in said *L. monocytogenes* isolate:
- 10 i. a gene, which is specific of *L. monocytogenes* clone CC1, wherein said CC1-specific gene is *LMOF2365_2702* (SEQ ID NO: 1),
 - ii. a gene, which is specific of *L. monocytogenes* clone CC2, wherein said CC2-specific gene is *LMOATCC19117_2417* (SEQ ID NO: 2),
 - iii. a gene, which is specific of *L. monocytogenes* clone CC4, wherein said CC4-specific
 - 15 gene is *LMO312_1102* (SEQ ID NO: 3),
 - iv. a gene, which is specific of *L. monocytogenes* clone CC6, wherein said CC6-specific gene is *LM1816_RS00050* (SEQ ID NO: 4),
 - v. a gene, which is specific of *L. monocytogenes* clone CC7, wherein said CC7-specific gene is *LMRG_02577* (SEQ ID NO: 9),
 - 20 vi. a gene, which is specific of *L. monocytogenes* clone CC3, wherein said CC3-specific gene is *LMOSLCC2482_0307* (SEQ ID NO: 5),
 - vii. a gene, which is specific of *L. monocytogenes* clone CC5, wherein said CC5-specific gene is *M637_08170* (SEQ ID NO: 6),
 - viii. a gene, which is specific of *L. monocytogenes* clones CC8 and CC16, wherein said
 - 25 CC8- and CC16-specific gene of vii. is *LM5578_1185* (SEQ ID NO: 10), and
 - ix. a gene, which is specific of *L. monocytogenes* clone CC155, wherein said CC155-specific gene is *LMLG_RS02400* (SEQ ID NO: 8),
- wherein the presence of one of said genes i.-ix. is indicative that said *L. monocytogenes* isolate has a high probability of belonging to the clone(s) that is(are) recited in i.-ix., respectively.
- 30
2. An *in vitro* method for determining that a *Listeria monocytogenes* isolate, which belongs to serogroup IVb or to serogroup IIb or to one of serogroups IIa and IIc, has a potentiality, more particularly a high potentiality, of causing invasive infection in a human, wherein said method comprises
- 35 when said *L. monocytogenes* isolate belongs to serogroup IVb, detecting that one of genes i.-iv. is present in said *L. monocytogenes* isolate,

when said *L. monocytogenes* isolate belongs to serogroup IIb, detecting that one of genes vi. and vii. is present in said *L. monocytogenes* isolate,

when said *L. monocytogenes* isolate belongs to one of serogroups IIa and IIc, detecting that one of genes v., viii. and ix. is present in said *L. monocytogenes* isolate;

- 5 said genes i.-iv., said genes vi. and vii., and said genes v., viii. and ix. being as defined in claim 1, wherein detecting that one of said genes i.-iv., or of said genes vi. and vii., or of said genes v., viii. and ix., is present in said *L. monocytogenes* isolate is indicative that said *L. monocytogenes* isolate has a potentiality, more particularly a high potentiality, of causing invasive infection in a human.

- 10 3. The *in vitro* method of claim 2, which further comprises, when said *L. monocytogenes* isolate belongs to one of serogroups IIa and IIc, determining whether one or none of the further following genes is or are present:

x. a gene, which is specific of *L. monocytogenes* clone CC121, wherein said CC121-specific gene is *LM6179_0610* (SEQ ID NO: 7); and

- 15 xi. a gene, which is specific of *L. monocytogenes* clone CC9, wherein said CC9-specific gene is *lmo1118* (SEQ ID NO: 11),

wherein detecting that one of said genes x. and xi., is present in said *L. monocytogenes* isolate is indicative that said *L. monocytogenes* isolate does not have a potentiality, more particularly a high potentiality, of causing invasive infection in a human, and

- 20 wherein detecting that one of said genes i.-iv., or of said genes vi. and vii., or of said genes v., viii. and ix., respectively, is present in said *L. monocytogenes* isolate is indicative that said *L. monocytogenes* isolate has a potentiality, more particularly a high potentiality, of causing invasive infection in a human.

- 25 4. The *in vitro* method of any one of claims 1-3, wherein detecting that one of said genes is present is performed by determining the presence or absence of a plurality of said genes.

5. The *in vitro* method of claim 4, wherein said plurality of genes comprises

- said genes i.-iv., or
- 30 - said genes vi.-vii., or
- said genes v., viii. and ix., or
- said genes v., and viii.-xi., or
- said genes i.-iv. and said genes vi.-vii., or
- said genes i.-iv. and said genes v., viii. and ix., or
- 35 - said genes i.-iv. and said genes v., and viii.-xi., or
- said genes vi.-vii. and said genes v., viii. and ix., or
- said genes vi.-vii. and said genes v., and viii.-xi., or

- said genes i.-iv., said genes vi.-vii. and said genes v., viii. and ix., or
- said genes i.-iv., said genes vi.-vii. and said genes v., and viii.-xi.

6. The *in vitro* method of claim 4 or 5, wherein primers and/or probes are used to determine the presence or absence of each gene of said plurality.

7. The *in vitro* method of claim 6, wherein said primers comprise

- a primer pair for specifically amplifying the sequence of SEQ ID NO: 16 from said gene i. of SEQ ID NO: 1, wherein said primer pair is
 - the primer pair of SEQ ID NOs: 17 and 18, or
 - a primer pair, which anneals to gene i. of SEQ ID NO: 1 to specifically amplify the sequence of SEQ ID NO: 16, wherein the first primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 17 and is of the same nucleotide length as SEQ ID NO: 17, and wherein the second primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 18 and is of the same nucleotide length as SEQ ID NO: 18;
- a primer pair for specifically amplifying the sequence of SEQ ID NO: 19 from said gene ii. of SEQ ID NO: 2, which is
 - the primer pair of SEQ ID NOs: 20 and 21, or
 - a primer pair, which anneals to gene ii. of SEQ ID NO: 2 to specifically amplify the sequence of SEQ ID NO: 19, wherein the first primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 20 and is of the same nucleotide length as SEQ ID NO: 20, and wherein the second primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 21 and is of the same nucleotide length as SEQ ID NO: 21;
- a primer pair for specifically amplifying the sequence of SEQ ID NO: 22 from said gene iii. of SEQ ID NO: 3, which is
 - the primer pair of SEQ ID NOs: 23 and 24, or
 - a primer pair, which anneals to gene iii. of SEQ ID NO: 3 to specifically amplify the sequence of SEQ ID NO: 22, wherein the first primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 23 and is of the same nucleotide length as SEQ ID NO: 23, and wherein the second primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 24 and is of the same nucleotide length as SEQ ID NO: 24; and
- a primer pair for specifically amplifying the sequence of SEQ ID NO: 25 from said gene iv. of SEQ ID NO: 4, which is
 - the primer pair of SEQ ID NOs: 26 and 27, or
 - a primer pair, which anneals to gene iv. of SEQ ID NO: 4 to specifically amplify the sequence of SEQ ID NO: 25, wherein the first primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 26 and is of the same nucleotide length as SEQ ID NO:

26, and wherein the second primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 27 and is of the same nucleotide length as SEQ ID NO: 27.

8. The *in vitro* method of claim 6, wherein said primers comprise

- 5 - a primer pair for specifically amplifying the sequence of SEQ ID NO: 28 from said gene vi. of SEQ ID NO: 5, which is
- the primer pair of SEQ ID NOs: 29 and 30, or
 - a primer pair, which anneals to gene vi. of SEQ ID NO: 5 to specifically amplify the sequence of SEQ ID NO: 28, wherein the first primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 29 and is of the same nucleotide length as SEQ ID NO: 29, and wherein the second primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 30 and is of the same nucleotide length as SEQ ID NO: 30; and
 - a primer pair for specifically amplifying the sequence of SEQ ID NO: 31 from said gene vii. of SEQ ID NO: 6, which is
- 15 - the primer pair of SEQ ID NOs: 32 and 33, or
- a primer pair, which anneals to gene vii. of SEQ ID NO: 6 to specifically amplify the sequence of SEQ ID NO: 31, wherein the first primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 32 and is of the same nucleotide length as SEQ ID NO: 32, and wherein the second primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 33 and is of the same nucleotide length as SEQ ID NO: 33.
- 20

9. The *in vitro* method of claim 6, wherein said primers comprise

- a primer pair for specifically amplifying the sequence of SEQ ID NO: 40 from said gene v. of SEQ ID NO: 9, which is
- 25 - the primer pair of SEQ ID NOs: 41 and 42, or
- a primer pair, which anneals to gene v. of SEQ ID NO: 9 to specifically amplify the sequence of SEQ ID NO: 40, wherein the first primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 41 and is of the same nucleotide length as SEQ ID NO: 41, and wherein the second primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 42 and is of the same nucleotide length as SEQ ID NO: 42;
- 30 - a primer pair for specifically amplifying the sequence of SEQ ID NO: 43 from said gene viii. of SEQ ID NO: 10, which is
- the primer pair of SEQ ID NOs: 44 and 45, or
 - a primer pair, which anneals to gene viii. of SEQ ID NO: 10 to specifically amplify the sequence of SEQ ID NO: 43, wherein the first primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 44 and is of the same nucleotide length as SEQ ID NO:
- 35

- 44, and wherein the second primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 45 and is of the same nucleotide length as SEQ ID NO: 45; and
- a primer pair for specifically amplifying the sequence of SEQ ID NO: 37 from said gene ix. of SEQ ID NO: 8, which is
- 5 - the primer pair of SEQ ID NOs: 38 and 39, or
- a primer pair, which anneals to gene ix. of SEQ ID NO: 8 to specifically amplify the sequence of SEQ ID NO: 37, wherein the first primer of said primer pair is at least 95% identical to the primer of SEQ ID NO: 38 and is of the same nucleotide length as SEQ ID NO: 38, and wherein the second primer of said primer pair is at least 95% identical to the primer of
- 10 SEQ ID NO: 39 and is of the same nucleotide length as SEQ ID NO: 39.

10. The *in vitro* method of any one of claims 4-9, wherein, in addition to determining the presence or absence of a plurality of said genes, said method further comprises determining the presence or absence of control marker gene(s), which is(are)
- 15 - a control marker gene or a combination of control marker genes, which is specific of *L. monocytogenes* serogroup IVb, and/or
- a control marker gene or a combination of control marker genes, which is specific of *L. monocytogenes* serogroup IIb, and/or
 - a control marker gene or a combination of control marker genes, which is specific of
- 20 *L. monocytogenes* serogroups IIa and IIc or of *L. monocytogenes* lineage II.

11. The *in vitro* method of any one of claims 7-9, wherein said primer pairs are mixed together.

12. The *in vitro* method of any one of claims 6-11, wherein said primers comprise
- 25 - the primers as defined in claim 7 and the primers as defined in claim 8, or
- the primers as defined in claim 7 and the primers as defined in claim 9, or
 - the primers as defined in claim 8 and the primers as defined in claim 9, or
 - the primers as defined in claim 7, the primers as defined in claim 8 and the primers as defined in claim 9,
- 30 and wherein said primers are implemented simultaneously under the same PCR thermocycling conditions.

13. A set of oligonucleotides, which comprises the primers as defined in claim 7 wherein said primers are in mixture in said set, or which comprises the primers as defined in claim 8 wherein said
- 35 primers are in mixture in said set, or which comprises the primers as defined in claim 9 wherein said primers are in mixture in said set.

14. A kit, which comprises

- the primers as defined in claim 7 and the primers as defined in claim 8, or which comprises
- the primers as defined in claim 7 and the primers as defined in claim 9, or which comprises
- the primers as defined in claim 8 and the primers as defined in claim 9, or which comprises
- 5 - the primers as defined in claim 7, the primers as defined in claim 8 and the primers as defined in claim 9.

15. The kit of claim 14, which comprises

- the primers as defined in claim 7 and the primers as defined in claim 8, wherein the primers as
10 defined in claim 7 are mixed together in a first set of oligonucleotides and/or the primers as defined in claim 8 are mixed together in a second set of oligonucleotides, or which comprises
- the primers as defined in claim 7 and the primers as defined in claim 9, wherein the primers as defined in claim 7 are mixed together in a first set of oligonucleotides and/or the primers as defined in claim 9 are mixed together in a second set of oligonucleotides, or which comprises
- 15 - the primers as defined in claim 8 and the primers as defined in claim 9, wherein the primers as defined in claim 8 are mixed together in a first set of oligonucleotides and/or the primers as defined in claim 9 are mixed together in a second set of oligonucleotides, or which comprises
- the primers as defined in claim 7, the primers as defined in claim 8 and the primers as defined in claim 9, wherein the primers as defined in claim 7 are mixed together in a first set of
20 oligonucleotides and/or the primers as defined in claim 8 are mixed together in a second set of oligonucleotides and/or the primers as defined in claim 9 are mixed together in a third set of oligonucleotides.

16. A computer device, which comprises

- 25 a processing unit in the memory of which is stored a computer program product for storage in a memory of a processing unit or on a removable memory support for cooperation with a reader of said processing unit and which further comprises
 - measurement values for the presence or absence of the genes as defined in any one of claims 1-12,
- 30 wherein said computer program product comprises instructions for determining that an isolate of *Listeria monocytogenes* has a high probability of belonging to clone CC1, to clone CC2, to clone CC4, to clone CC6, to clone CC7, to clone CC3, to clone CC5, to clone CC8 or CC16, or to clone CC155 of *L. monocytogenes*, wherein a high probability of belonging to one of these clones is indicative that said *L. monocytogenes* isolate has a
35 potentiality, more particularly a high potentiality, of causing invasive infection in a human, wherein the presence of one of said genes, as defined in any one of claims 1-12, is indicative that said *L. monocytogenes* isolate has a high probability of belonging to clone CC1, clone CC2, clone CC4,

clone CC6, clone CC7, clone CC3, clone CC5, clones CC8 and CC16, or clone CC155, respectively, or

- instructions for determining that a *Listeria monocytogenes* isolate, which belongs to serogroup IVb or to serogroup IIb or to one of serogroups IIa and IIc, has a potentiality, more particularly a high potentiality, of causing invasive infection in a human, wherein the presence of one of said genes, as
- 5 defined in any one of claims 1-12, is indicative that said *L. monocytogenes* isolate has a potentiality, more particularly a high potentiality, of causing invasive infection in a human.

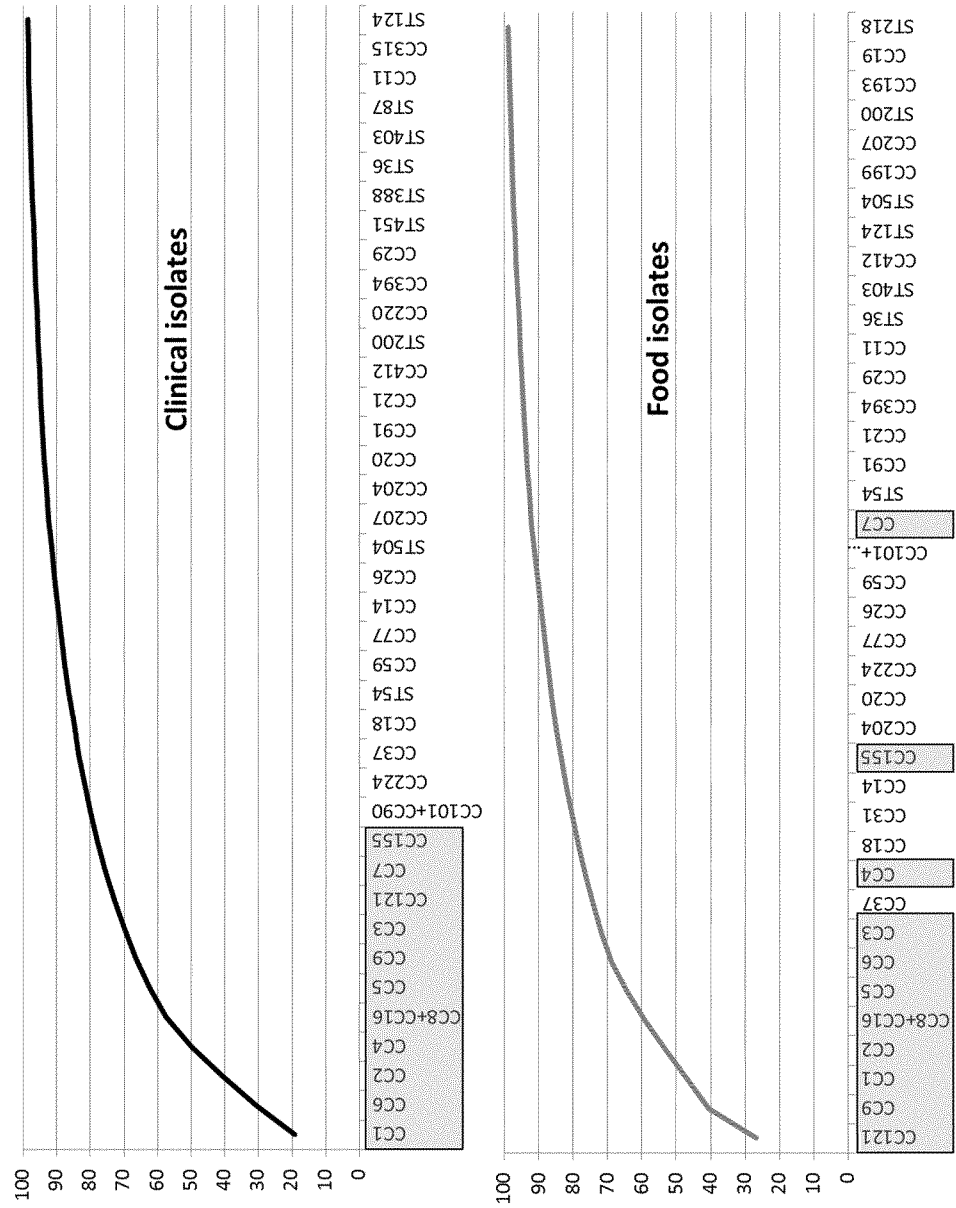
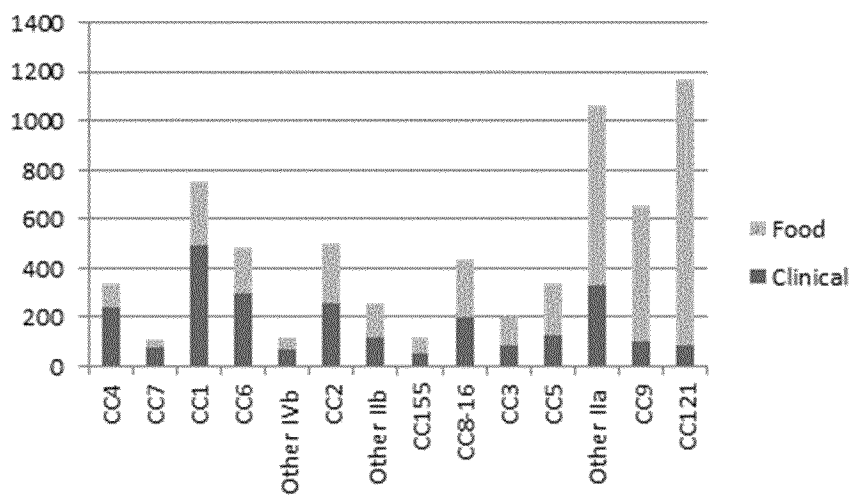
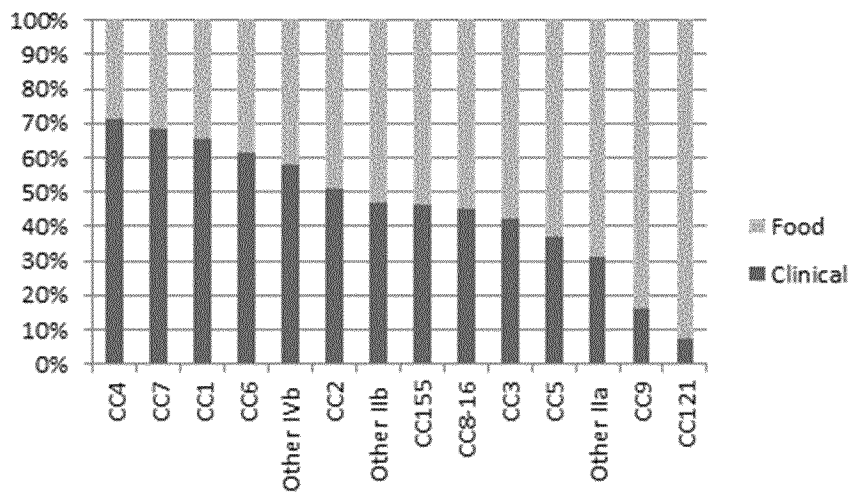


FIGURE 1

2/7



FIGURES 2A and 2B

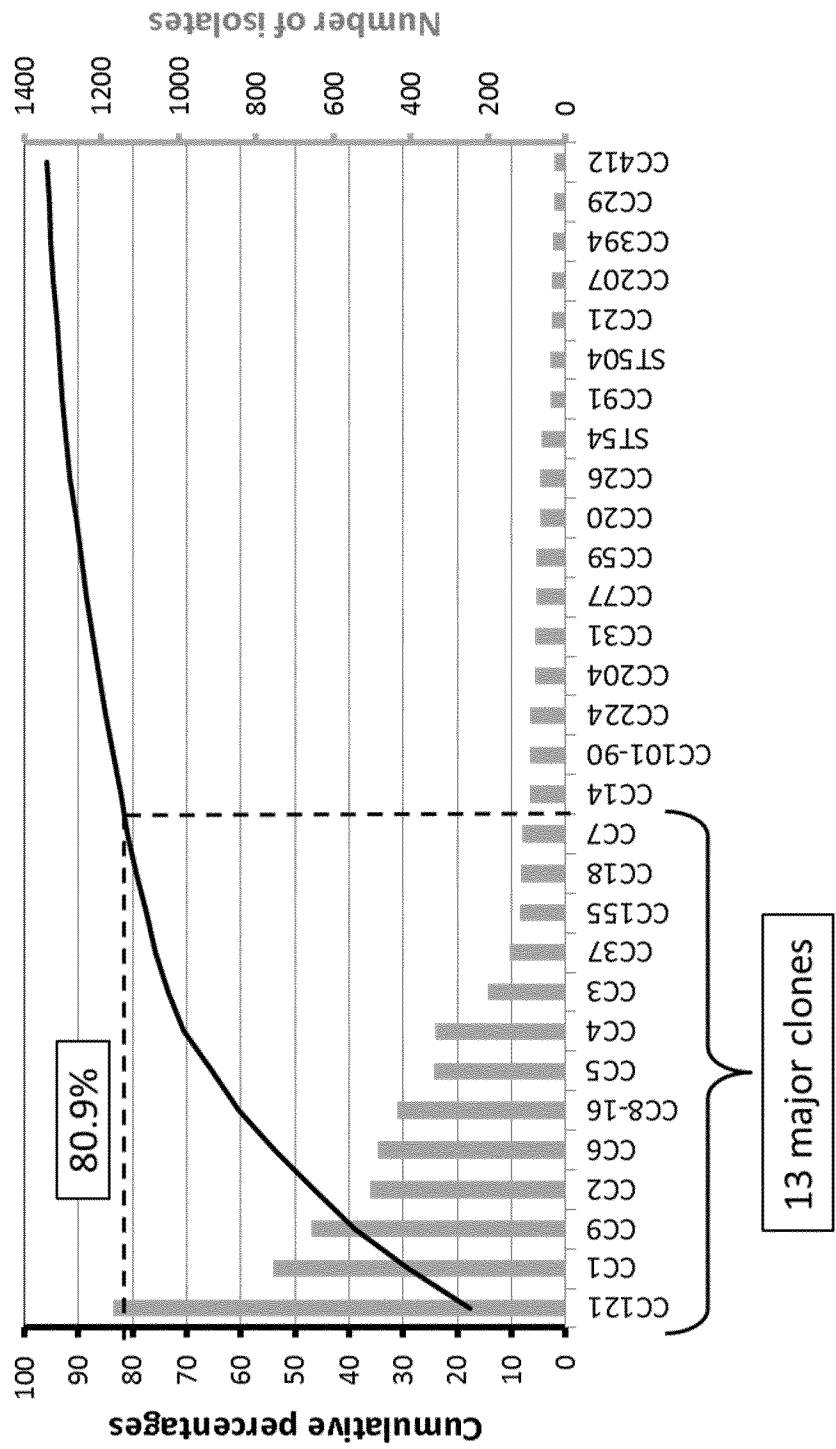
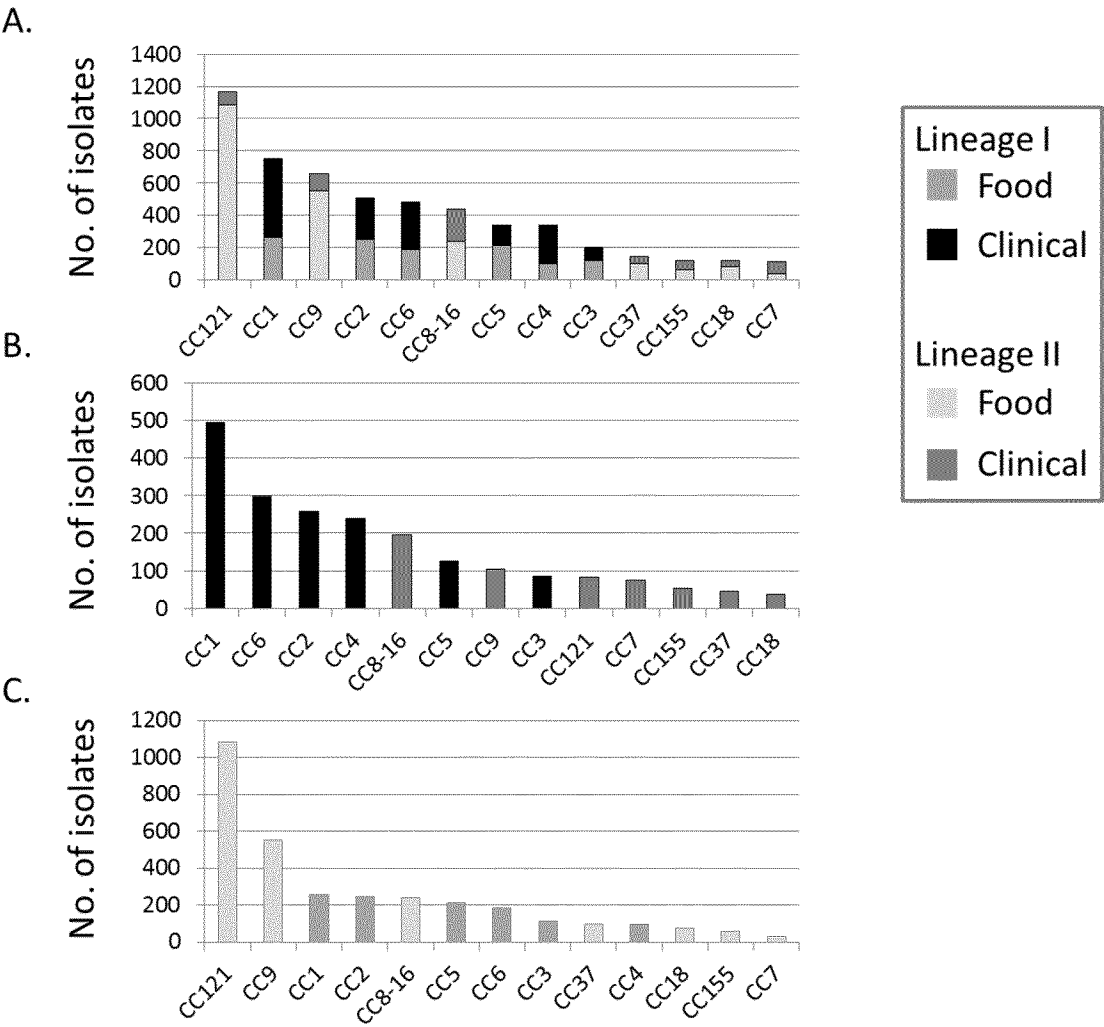


FIGURE 3



FIGURES 4A, 4B and 4C

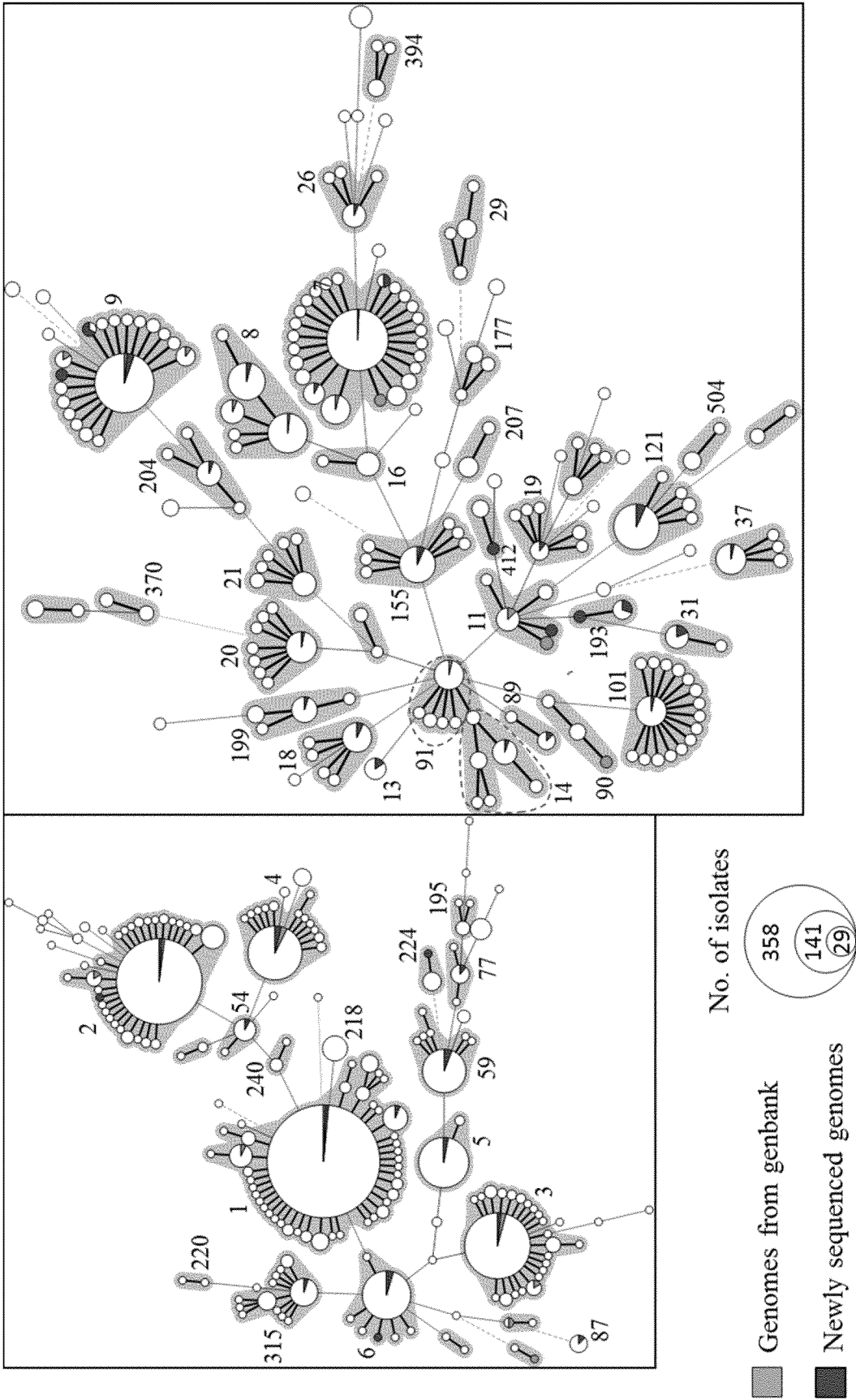
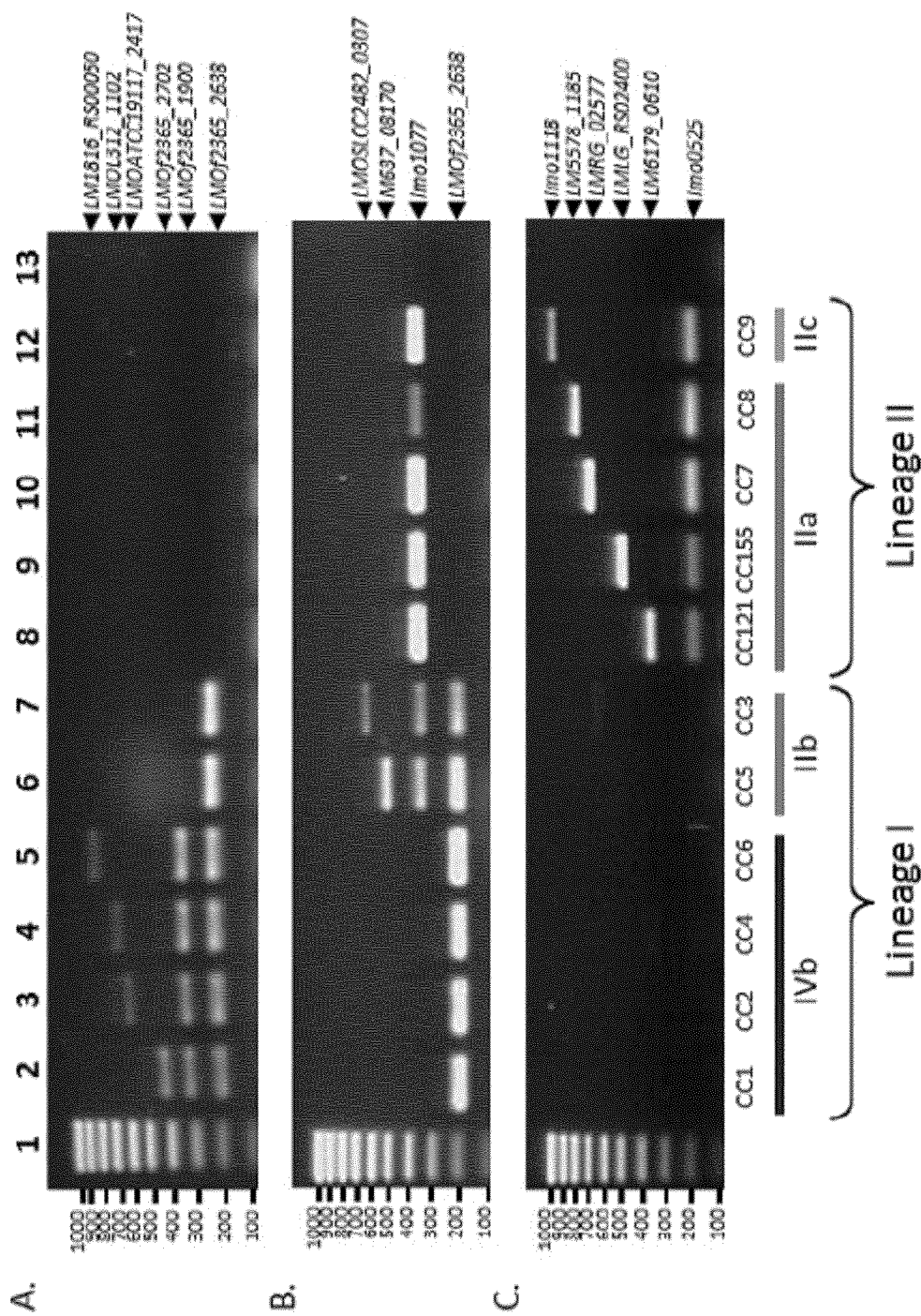


FIGURE 5



FIGURES 6A, 6B and 6C

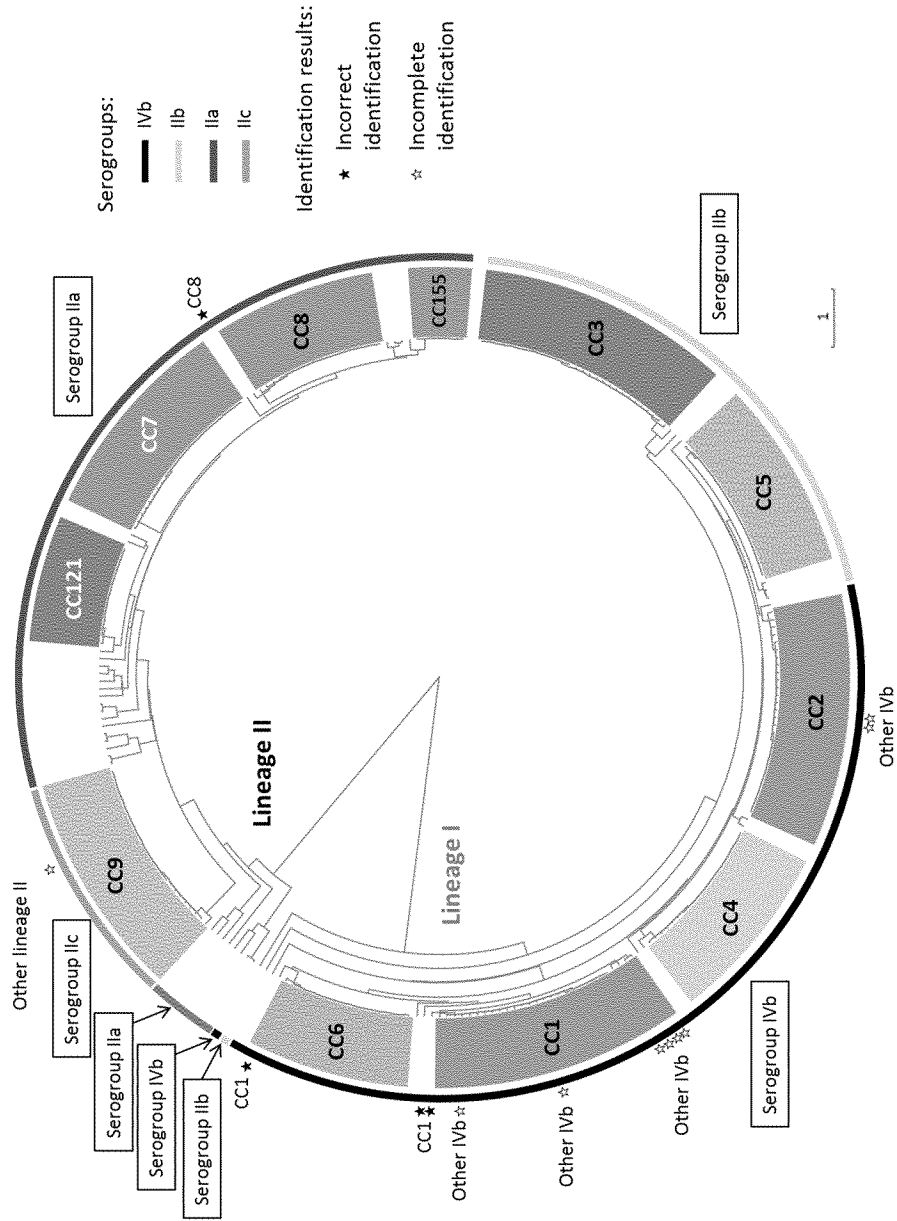


FIGURE 7

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/066209

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, CHEM ABS Data, EMBASE, FSTA, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2006/257894 A1 (DOUMITH MICHEL [FR] ET AL) 16 November 2006 (2006-11-16)	2,13-15
Y	claims 10 and 11; SEQ ID NO:15 in Table 3 on page 15 SEQ ID NO: 353 and 354, Table 5 on page 57 -----	3-12
Y	THOMAS CANTINELLI ET AL: "Epidemic Clones" of <i>Listeria monocytogenes</i> Are Widespread and Ancient Clonal Groups", JOURNAL OF CLINICAL MICROBIOLOGY, vol. 51, no. 11, 4 September 2013 (2013-09-04), pages 3770-3779, XP055227153, US	3-12
A	ISSN: 0095-1137, DOI: 10.1128/JCM.01874-13 page 3770, right col. ----- -/--	1

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* 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

"T" 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

"X" 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

"&" document member of the same patent family

Date of the actual completion of the international search

18 August 2016

Date of mailing of the international search report

26/10/2016

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

Leber, Thomas

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2016/066209

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13~~ter~~.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/066209

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	VIVIANE CHENAL-FRANCISQUE ET AL: "Worldwide Distribution of Major Clones of Listeria monocytogenes", EMERGING INFECTIOUS DISEASES, vol. 17, no. 6, 1 June 2011 (2011-06-01), pages 1110-1112, XP055227157, US ISSN: 1080-6040, DOI: 10.3201/eid1706.101778	3-12
A	page 1110, right col.	1
Y	MARIE RAGON ET AL: "A New Perspective on Listeria monocytogenes Evolution", PLOS PATHOGENS, vol. 4, no. 9, 5 September 2008 (2008-09-05), page e1000146, XP055227513, DOI: 10.1371/journal.ppat.1000146	3-12
A	page 2, right col.; table 1	1
A	M. DOUMITH ET AL: "Differentiation of the Major Listeria monocytogenes Serovars by Multiplex PCR", JOURNAL OF CLINICAL MICROBIOLOGY, vol. 42, no. 8, 1 August 2004 (2004-08-01) , pages 3819-3822, XP055227159, US ISSN: 0095-1137, DOI: 10.1128/JCM.42.8.3819-3822.2004 Abstract; page 3819, right col.; Fig. 1	1-16
A	CARSTEN KUENNE ET AL: "Reassessment of the Listeria monocytogenes pan-genome reveals dynamic integration hotspots and mobile genetic elements as major components of the accessory genome", BMC GENOMICS, BIOMED CENTRAL LTD, LONDON, UK, vol. 14, no. 1, 22 January 2013 (2013-01-22), page 47, XP021134819, ISSN: 1471-2164, DOI: 10.1186/1471-2164-14-47 Table 1	1-16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2016/066209

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of Item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-16(partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-16(partially)

Methods, set of oligonucleotides, kit and computer device suitable for detecting *Listeria monocytogenes* of serogroup IVb.

2. claims: 1-16(partially)

Methods, set of oligonucleotides, kit and computer device suitable for detecting *Listeria monocytogenes* of serogroup IIb.

3. claims: 1-16(partially)

Methods, set of oligonucleotides, kit and computer device suitable for detecting *Listeria monocytogenes* of serogroup IIa or IIc.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2016/066209

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2006257894	A1	16-11-2006	NONE
