

SUMMARY

In one example, the present disclosure refers to a method for the detection of rhamnose fermenting *Listeria* spp., such as *Listeria monocytogenes*, in a sample, said method

5 comprising or consisting of the steps of:

- i) preparing a suspension of a sample potentially containing *Listeria* bacteria in a first culture medium, said first culture medium comprising rhamnose, one or more of an antibiotic, a pH colour indicator and LiCl;
- 10 ii) incubating said suspension at conditions allowing the growth of *Listeria* bacteria; and
- 10 iii) identifying positive samples.

In one aspect, the present disclosure refers to a method for the detection of rhamnose fermenting *Listeria* spp., such as *Listeria monocytogenes*, in a sample, said method comprising or consisting of the steps of:

- 15 i) preparing a suspension of a sample potentially containing *Listeria* bacteria in a first culture medium, said first culture medium comprising:
 - rhamnose;
 - antibiotics, wherein said antibiotics are three, four or all five of nalidixic acid, ceftazidime, polymyxin B sulfate, cycloheximide and
 - 20 amphotericin B;
 - a pH colour indicator; and
 - LiCl;
- ii) incubating said suspension of the sample in the first culture medium at conditions allowing the growth of *Listeria* bacteria; and
- 25 iii) identifying positive samples.

The method may further comprise the enumeration of rhamnose fermenting *Listeria* spp., wherein the method comprises a step ia) of transferring the suspension prepared in step i) to a multi-well tray and a step iv) performed after step iii) of calculating the concentration of said *Listeria* in said sample, such as by using the Most Probable Number method.

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The method for enumeration of the number of rhamnose fermenting *Listeria* spp., such as *Listeria monocytogenes*, thus comprise or consistsof the steps of:

- i) preparing a suspension of a sample potentially containing *Listeria* bacteria in a first culture medium comprising rhamnose, one or more of an antibiotic, a pH colour indicator and LiCl;
- 35 ia) transferring the suspension obtained in step i) to a multi-well tray;
- ii) incubating said suspension at conditions allowing the growth of *Listeria* bacteria;
- iii) identifying positive samples; and
- 40 iv) calculating the concentration of said *Listeria* in said sample, such as by using the Most Probable Number method.

The method of the present document may further include a step of confirming the presence of *Listeria monocytogenes* by distinguishing *Listeria monocytogenes* from other

45 *Listeria* species, such as by plating positive samples identified in step iii) on a second growth medium such as ALOA medium, by utilizing a molecular method, such as by polymerase chain reaction or in situ hybridization ELISA (enzyme-linked immunosorbent

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- a) rhamnose in a concentration of about 5-15 g/l, such as about 7-13 g/l, such as about 10 g/l;
- b) one or more antibiotics, such as nalidixic acid, ceftazidime, polymyxin B sulfate, cycloheximide, amphotericine B, preferably at least two of these;
- 5 c) a pH colour indicator, such as phenol red; and
- d) LiCl in a concentration of about 5-17 g/l, such as about 7-13 g/l, such as about 10 g/l.

In one aspect, the present disclosure refers to a culture medium for the growth and/or detection of rhamnose fermenting *Listeria* spp., such as *Listeria monocytogenes*, said culture medium comprising:

- 10 a) rhamnose in a concentration of about 5-15 g/l, such as about 7-13 g/l, such as about 10 g/l;
- b) antibiotics, wherein said antibiotics are three, four or all five of nalidixic acid, ceftazidime, polymyxin B sulfate, cycloheximide, and amphotericin B;
- 15 c) a pH colour indicator, such as phenol red; and
- d) LiCl in a concentration of about 5 to about 15 g/l, such as about 7 to about 13 g/l, such as about 10 g/l.

In one example, the present disclosure also refers to a kit for detecting and/or enumerating rhamnose fermenting *Listeria* spp., such as *Listeria monocytogenes*, in a sample, said kit comprising:

- a) a container comprising a culture medium comprising or consisting of rhamnose, one or more of an antibiotic, a pH colour indicator and LiCl;
- 25 b) a container or multi-well tray for cultivating a sample possibly containing *Listeria* bacteria;
- c) optionally a colour chart for identifying positive samples; and
- d) optionally instructions for use.

The concentrations of the constituents of the culture medium and the culture medium of the kit are the same as the concentrations of the constituents in the first culture medium described herein.

In one aspect, the present disclosure refers to a kit for detecting and/or enumerating rhamnose fermenting *Listeria* spp., such as *Listeria monocytogenes*, in a sample, said kit comprising:

- 35 a) a container comprising a culture medium comprising or consisting of:
rhamnose;
antibiotics, wherein said antibiotics are three, four or all five of nalidixic acid, ceftazidime, polymyxin B sulfate, cycloheximide and amphotericin B;
a pH colour indicator; and
LiCl;
- 40 b) a container or multi-well tray for cultivating a sample possibly containing *Listeria* bacteria; and
- c) optionally a colour chart for identifying positive samples; and
- d) optionally instructions for use.

In one aspect, the present disclosure also refers to a computer implemented calculator for displaying a prediction of the growth of *Listeria*, such as *Listeria monocytogenes*, based on the detection and enumeration of colony forming units in a sample by the method according to the present document, said calculator comprising:

- a) an input device, such as a keyboard or a microphone;
 - b) an output device, such as a display, a computer or mobile phone screen or a loudspeaker;
 - 50 c) software, downloadable or in memory, such as an app on a smart phone or a webpage;
- where the software accepts the input of any number of colony-forming units found in the sample in the range of 0.04 cfu/g to 1 cfu/g.

CLAIMS

1. A method for the detection of rhamnose fermenting *Listeria* spp., such as *Listeria monocytogenes*, in a sample, said method comprising or consisting of the steps of:
 - i) preparing a suspension of a sample potentially containing *Listeria* bacteria in a first culture medium, said first culture medium comprising:
 - rhamnose;
 - antibiotics, wherein said antibiotics are three, four or all five of nalidixic acid, ceftazidime, polymyxin B sulfate, cycloheximide and amphotericin B;
 - a pH colour indicator; and
 - LiCl;
 - ii) incubating said suspension of the sample in the first culture medium at conditions allowing the growth of *Listeria* bacteria; and
 - iii) identifying positive samples.
2. A method according to claim 1, said method further comprising the enumeration of rhamnose fermenting *Listeria* spp., said method comprising a step ia) of transferring the suspension of the sample in the first culture medium prepared in step i) to a multi-well tray and a step iv) being performed after step iii) of calculating the concentration of said *Listeria* in said sample, such as by using the Most Probable Number method.
3. The method according to claim 1 or 2, further including a step of confirming the presence of *Listeria monocytogenes*, such as by plating positive samples identified in step iii) on a second growth medium such as ALOA medium, by utilizing a molecular method, such as by polymerase chain reaction or *in situ* hybridization, ELISA, VITEC, and/or API, said step being performed after step iii) in claim 1 or step iv) in claim 2.
4. The method according to any one of the preceding claims, wherein said sample possibly containing rhamnose fermenting *Listeria* spp. is obtained by dividing said sample into small pieces such as by homogenization, slicing and/or chopping either before step i) is performed and/or in the first culture medium of step i).

5. The method according to any one of the preceding claims wherein said antibiotics are four or all five of nalidixic acid, ceftazidime, polymyxin B sulfate, cycloheximide and amphotericin B.
- 5 6. A method according to any one of the preceding claims wherein said pH colour indicator is phenol red.
7. A method according to any one of the preceding claims wherein said LiCl is present in an amount of about 5-17 g/l, such as about 7-13 g/l, such as about 10
10 g/l.
8. A method according to any one of the preceding claims wherein said rhamnose is present in an amount of about 5-17 g/l, such as about 7-13 g/l, such as about 10
15 g/l.
9. The method according to any one of the preceding claims, wherein said sample is a food sample, an environmental sample, or a sample from an animal, such as a human, such as a tissue sample or a feces sample.
- 20 10. The method according to claim 9, wherein said food sample is raw or processed meat, poultry or fish products, vegetables or a ready-to-eat food product.
11. The method according to claim 9, wherein said environmental sample is a water sample, a dirt sample or a food industry environmental sample, such as a surface
25 swab sample.
12. The method according to any one of the preceding claims, wherein said method is performed in a closed system.
- 30 13. A culture medium for the growth and/or detection of rhamnose fermenting *Listeria* spp., such as *Listeria monocytogenes*, said culture medium comprising:
 - a) rhamnose in a concentration of about 5-15 g/l, such as about 7-13 g/l, such as about 10 g/l;
 - 35 b) antibiotics, wherein said antibiotics are three, four or all five of nalidixic acid, ceftazidime, polymyxin B sulfate, cycloheximide and amphotericin B;

- c) a pH colour indicator, such as phenol red; and
- d) LiCl in a concentration of about from 5 to about 15 g/l, such as about from 7 to about 13 g/l, such as about 10 g/l.

- 5 14. A kit for detecting and/or enumerating rhamnose fermenting *Listeria* spp., such as *Listeria monocytogenes*, in a sample, said kit comprising:
 - a) a container comprising a culture medium comprising or consisting of:
 - rhamnose;
 - antibiotics, wherein said antibiotics are three, four or all five of nalidixic acid,
 - 10 ceftazidime, polymyxin B sulfate, cycloheximide and amphotericin B;
 - a pH colour indicator; and
 - LiCl;
 - b) a container or multi-well tray for cultivating a sample possibly containing *Listeria* bacteria; and
 - 15 c) optionally a colour chart for identifying positive samples; and
 - d) optionally instructions for use.

- 20 15. A computer implemented calculator for displaying a prediction of the growth of *Listeria*, such as *Listeria monocytogenes*, based on the detection and enumeration of colony forming units in a sample by the method according to any one of claims 1-12, said calculator comprising:
 - a) an input device, such as a keyboard or a microphone;
 - b) an output device, such as a display, a computer or mobile phone screen or a loudspeaker;
 - 25 c) software, downloadable or in memory, such as an app on a smart phone or a webpage;
 - where the software accepts the input of any number of colony-forming units found in the sample in the range of 0.04 cfu/g to 1 cfu/g.