

## SUMMARY

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

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;
- ii) incubating said suspension at conditions allowing the growth of *Listeria*  
10 bacteria; and
- 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  
15 of said *Listeria* in said sample, such as by using the Most Probable Number method.

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

- 20 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;
- ia) transferring the suspension obtained in step i) to a multi-well tray;
- ii) incubating said suspension at conditions allowing the growth of *Listeria*  
25 bacteria;
- iii) identifying positive samples; and
- iv) calculating the concentration of said *Listeria* in said sample, such as by using the Most Probable Number method.

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

- 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) one or more antibiotics, such as nalidixic acid, ceftazidime, polymyxin B sulfate, cycloheximide, amphotericine B, preferably at least two of these;
- 15 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.

In one aspect, 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;
- b) a container or multi-well tray for cultivating a sample possibly containing *Listeria* bacteria;
- 25 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 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:

- 35 a) an input device, such as a keyboard or a microphone;
- a) an output device, such as a display, a computer or mobile phone screen or a loudspeaker;
- b) software, downloadable or in memory, such as an app on a smart phone or a webpage;
- 40 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.

Other features and advantages of the disclosure will be apparent from the following detailed description, drawings, examples, and from the claims.

## DEFINITIONS

5 Most Probable Number (MPN) is a method used to estimate the concentration of viable microorganisms in a sample by means of replicate liquid broth growth in x-fold dilutions (such as 15x, 10x or 2x dilutions). Most Probable Number may be used for estimating microbial populations in soils, waters, agricultural products and is particularly useful with samples that contain particulate material that interferes with plate count enumeration  
10 methods.

A colony-forming unit (cfu) is a unit used to estimate the number of viable bacteria or fungal cells in a sample. Viable is defined as the ability to multiply via binary fission under controlled conditions. Counting with colony-forming units requires culturing the microbes  
15 and counts only viable cells. The visual appearance of a colony in a cell culture requires significant growth.

“Cultivating”, “cultivation” and the like refers to growing or maintaining living cells in a growth or culture medium which is solid or liquid designed to support the growth of  
20 microorganisms or cells. The growth or culture medium comprises nutrients essential and relevant for the intended respective microorganisms or cells to grow.

ALOA (Agar medium for *Listeria* after Ottaviani and Augusti) medium is a selective chromogenic agar medium for the selective and differential isolation of *Listeria*  
25 *monocytogenes*. The medium is described in the ISO method.

Polymerase Chain Reaction, PCR, is a method of making multiple copies of a DNA sequence, involving repeated reactions with a polymerase.

30 *In situ* hybridization, ISH, is a technique that allows for precise localization of a specific segment of nucleic acid within a histologic section. The underlying basis of ISH is that nucleic acids, if preserved adequately within a histologic specimen, can be detected through the application of a complementary strand of nucleic acid to which a reporter molecule is attached.

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