INTERNATIONAL STANDARD

ISO 11290-2

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AMENDMENT 1
2004-10-15

Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of *Listeria monocytogenes* —

Part 2: Enumeration method

AMENDMENT 1: Modification of the enumeration medium

Microbiologie des aliments — Méthode horizontale pour la recherche et le dénombrement de Listeria monocytogenes —

Partie 2: Méthode de dénombrement

AMENDEMENT 1: Modification du milieu d'isolement

Reference number
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ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

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Foreword

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Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

Amendment 1 to ISO 11290-2:1998 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

The enumeration medium has been modified.

Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of *Listeria monocytogenes* —

Part 2:
Enumeration method

AMENDMENT 1: Modification of the enumeration medium

Throughout the whole text of this part of ISO 11290:

— Change “PALCAM” to “Agar *Listeria* according to Ottaviani and Agosti”.

— Change the incubation temperature to 37 °C. (No choice is given between 35 °C and 37 °C.)

Page 3

Replace item 6.4 by the following:

6.4 Water bath, capable of being maintained at 44 °C to 47 °C.

Page 4, Subclause 9.2.3

Delete the 2nd sentence.

Page 5, Subclauses 9.3.2 and 9.3.3

Delete these subclauses and replace them by the following:

9.3.2 Consider as *L. monocytogenes* the green-blue colonies surrounded by an opaque halo (typical colonies). If growth is slight, or if no colony is observed, or if no typical colony is present after 24 h ± 3 h of incubation, re-incubate the plates for a further 24 h ± 3 h.

NOTE 1 Some strains of *L. monocytogenes* show a very weak halo (even no halo) in cases of stress, in particular acid stress.

NOTE 2 Some *L. monocytogenes* are characterized by a slow PIPLC (phosphatidyl inositol phospholipase C) activity. Such bacteria are detected when the total duration of incubation is more than, for example, 4 days. Some of these strains could be pathogenic (see Reference [1]).

Renumber 9.3.4 as 9.3.3.

Page 13, Clause B.3

Replace the existing clause by the following:

B.3 Agar *Listeria* according to Ottaviani and Agosti (ALOA [1])

B.3.1 Base medium

B.3.1.1 Composition

Enzymatic digest of animal tissues	18 g
Enzymatic digest of casein	6 g
Yeast extract	10 g
Sodium pyruvate	2 g
Glucose	2 g
Magnesium glycerophosphate	1 g
Magnesium sulfate (anhydrous)	0,5 g
Sodium chloride	5 g
Lithium chloride	10 g
Disodium hydrogen phosphate (anhydrous)	2,5 g
5-Bromo-4-chloro-3-indolyl-β-D-glucopyranoside	0,05 g
Agar	12 g to 18 g [a]
Water	930 ml [b]

[a] Depending on the gel strength of the agar.

[b] 925 ml if Amphotericin B solution is used (see B.3.5.2).

B.3.1.2 Preparation

Dissolve the dehydrated components or dehydrated complete base in the water by boiling.

Sterilize for 15 min in the autoclave set at 121 °C.

Adjust the pH, if necessary, so that after sterilization it is 7,2 ± 0,2.

B.3.2 Nalidixic acid solution

Nalidixic acid sodium salt	0,02 g
Sodium hydroxide (0,05 mol/l)	5 ml

Dissolve the nalidixic acid sodium salt in 5 ml of sodium hydroxide and sterilize by filtration.

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1) ALOA is an example of a suitable medium available commercially. This information is given for the convenience of users of this part of ISO 11290 and does not constitute an endorsement by ISO of this product. The use of other media with the same formulation is allowed.

B.3.3 Ceftazidime solution

Ceftazidime	0,02 g
Water	5 ml

Dissolve the ceftazidime in 5 ml of water and sterilize by filtration through a 0,45 µm membrane.

B.3.4 Polymyxin B solution

Polymyxin B sulfate	76 700 IU
Water	5 ml

Dissolve the polymyxin B in 5 ml of water. Sterilize by filtration through a 0,45 µm membrane.

B.3.5 Antibiotic supplement

B.3.5.1 Cycloheximide solution

Cycloheximide	0,05 g
Ethanol	2,5 ml
Water	2,5 ml

Dissolve the cycloheximide in 2,5 ml of ethanol then add 2,5 ml of water. Sterilize by filtration through a 0,45 µm membrane.

B.3.5.2 **Amphotericin B solution** (as an alternative to cycloheximide solution)

Amphotericin B	0,01 g
HCl (1 mol/l)	2,5 ml
Dimethylformamide (DMF)	7,5 ml

Dissolve the amphotericin in the HCl/DMF solution. Sterilize by filtration through a 0,45 µm membrane.

WARNING — The HCl/DMF solution is toxic, handle with care.

B.3.6 Supplement

Dissolve 2 g of L-α-phosphatidylinositol (Sigma P 6636® [2]) in 50 ml of cold water.

Stir for about 30 min until a homogeneous suspension is obtained.

Autoclave at 121 °C for 15 min and cool to 48 °C to 50 °C.

2) P 6636 is a trade name of a product supplied by Sigma. This information is given for the convenience of the users of this part of ISO 11290 and does not constitute an endorsement by ISO of this product. Equivalent products may be used if they can be shown to lead to the same results.

B.3.7 Complete medium

B.3.7.1 Composition

Base medium (B.3.1)	930 ml [a]
Nalidixic acid solution (B.3.2)	5 ml
Ceftazidime solution (B.3.3)	5 ml
Polymyxin B solution (B.3.4)	5 ml
Cycloheximide solution (B.3.5.1)	5 ml
or Amphotericin B solution (B.3.5.2)	10 ml
Supplement (B.3.6)	50 ml

[a] 925 ml if Amphotericin B solution is used.

B.3.7.2 Preparation

Add the solutions to the molten base at 50 °C, mixing thoroughly between each addition.

The pH of the complete medium shall be 7,2 ± 0,2 at 25 °C.

The medium shall be homogeneously opaque.

B.3.7.3 Preparation of agar plates

Place in each Petri dish 15 ml to 20 ml of the freshly prepared complete medium, then allow to solidify.

Add a Bibliography after Annex B.

Bibliography

[1] LECLERCQ, A. Colonial atypical morphology and low recoveries of *Listeria monocytogenes* strains on Oxford, PALCAM, Rapid'L.mono and ALOA solid media. *J. Microbiol. Methods*, **57**, 2004, pp. 251-258