

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

Part 1: Detection method

ICS 07.100.30

National foreword

This British Standard is the UK implementation of EN ISO 11290-1:1996 +A1:2004. It is identical with ISO 11290-1:1996, incorporating amendment 1:2004. Together with BS EN ISO 11290-2:1998+A1:2004, it supersedes BS 4285-3.15:1993 (ISO 10560:1993) which is withdrawn.

The start and finish of text introduced or altered by amendment is indicated in the text by tags . Tags indicating changes to ISO text carry the number of the ISO amendment. For example, text altered by ISO amendment 1 is indicated by A1 A1.

The UK participation in its preparation was entrusted to Technical Committee AW/9, Microbiology.

A list of organizations represented on this committee can be obtained on request to its secretary.

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(ISO 11290-1:1996 + A1:2004)

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Foreword

The text of the International Standard ISO 11290-1:1996 has been prepared by Technical Committee ISO/TC 34, *Agricultural food products*, in collaboration with Technical Committee CEN/TC 275, *Food analysis — Horizontal methods*, the Secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by June 1997, and conflicting national standards shall be withdrawn at the latest by June 1997.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

Endorsement notice

The text of the International Standard ISO 11290-1:1996 was approved by CEN as a European Standard without any modification.

Foreword to amendment A1

This document (EN ISO 11290-1:1996/A1:2004) has been prepared by Technical Committee ISO/TC 34, *Agricultural food products*, in collaboration with Technical Committee CEN/TC 275, *Food analysis — Horizontal methods*, the Secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by April 2005, and conflicting national standards shall be withdrawn at the latest by April 2005.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Slovakia, Slovenia, Spain, Sweden, Switzerland and the United Kingdom.

Endorsement notice

The text of ISO 11290-1:1996 has been approved by CEN as EN ISO 11290-1:1996/A1:2004 without any modifications.

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Introduction

Because of the large variety of food and feed products, this horizontal method may not be appropriate in every detail for certain products for which it may be necessary to use different or specific methods. Nevertheless, in all cases, every attempt should be made to apply this horizontal method as far as possible and that deviations from this will only be made if absolutely necessary for justified technical reasons.

When this part of ISO 11290 is next reviewed, account will be taken of all information then available regarding the extent to which this horizontal method has been followed and the reasons for deviations from it in the case of particular products.

The harmonization of test methods cannot be immediate, and for certain groups of products International Standards and/or national standards may already exist that do not comply with this horizontal method. It is hoped that when such standards are reviewed they will be changed to comply with this part of ISO 11290 so that eventually the only remaining departures from this horizontal method will be those necessary for well-established technical reasons.

WARNING — In order to safeguard the health of laboratory personnel, it is strongly recommended that tests for detecting *Listeria monocytogenes* are undertaken in properly equipped laboratories, under the control of a skilled microbiologist, and that great care is taken in the disposal of all incubated materials. In particular, it is strongly recommended that pregnant personnel do not manipulate cultures of *L. monocytogenes*.

1 Scope

This part of ISO 11290 specifies a horizontal method for the detection of *Listeria monocytogenes*.

Subject to the limitations discussed in the introduction, this part of ISO 11290 is applicable to products intended for human consumption or animal feeding.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this part of ISO 11290. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this part of ISO 11290 are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 6887:1983, *Microbiology — General guidance for the preparation of dilutions for microbiological examination*.

ISO 7218:1996, *Microbiology of food and animal feeding stuffs — General rules for microbiological examinations*.

3 Definitions

For the purposes of this part of ISO 11290, the following definitions apply.

3.1

Listeria monocytogenes

microorganisms which form typical colonies on solid selective media and which display the morphological, physiological and biochemical characteristics described when tests are carried out in accordance with this part of ISO 11290

3.2

detection of *Listeria monocytogenes*

determination of the presence or absence of these microorganisms, in a given mass or volume of product, when tests are carried out in accordance with this part of ISO 11290

4 Principle

Within the limits of this part of ISO 11290, the detection of *L. monocytogenes* necessitates four successive stages (see Annex A for a flowchart).

NOTE 1 *Listeria* spp. may be present in small numbers and are often accompanied by considerably larger numbers of other genera, therefore selective enrichment is necessary. It is also necessary to detect injured *Listeria* spp. and the primary selective enrichment medium, with reduced inhibitor concentration, fulfils at least part of this function.

4.1 Primary enrichment in a selective liquid enrichment medium with reduced concentration of selective agents (half Fraser broth)

Inoculation of a selective primary enrichment medium containing one volume of lithium chloride and half a volume of both acriflavine and nalidixic acid (half Fraser broth), which is also used as a dilution fluid for the test portion (9.1).

Incubation of the test portion at 30 °C for 24 h.

4.2 Secondary enrichment with a selective liquid enrichment medium with full concentration of selective agents (Fraser broth)

Inoculation of full-strength secondary liquid enrichment medium (Fraser broth) with a culture obtained from 4.1.

Incubation of the Fraser broth at 35 °C or 37 °C for 48 h.

A1 4.3 Plating out and identification

From the cultures obtained in 4.1 and 4.2, plating out on the two selective solid media:

- a) Agar *Listeria* according to Ottaviani and Agosti (ALOA¹⁾) (see Reference [1] and B.3);
- b) any other solid selective medium at the choice of the laboratory complementary to Agar *Listeria* according to Ottaviani and Agosti, such as Oxford or PALCAM.

Incubation of the Agar *Listeria* according to Ottaviani and Agosti at 37 °C ± 1 °C and examination after 24 h ± 3 h, and if necessary after a further 24 h ± 3 h, to check for the presence of characteristic colonies which are presumed to be *L. monocytogenes*.

Incubation of the 2nd selective medium at the appropriate temperature and examination after the appropriate time. **A1**

4.4 Confirmation

Subculturing of the colonies of presumptive *L. monocytogenes*, plated out as described in 4.3, and confirmation by means of appropriate morphological, physiological and biochemical tests.

5 Culture media and reagents

5.1 General

For current laboratory practice, see ISO 7218.

NOTE 2 Because of the large number of culture media and reagents, it has been considered preferable, for clarity of the text, to give their composition and preparation in Annex B.

5.2 Selective primary enrichment medium: Fraser broth with reduced concentration of selective agents (half Fraser broth)

See Clause B.1.

5.3 Selective secondary enrichment medium with full concentration of selective agents (Fraser broth)

See Clause B.2.

5.4 Selective solid plating-out media

A1 5.4.1 First medium: Agar *Listeria* according to Ottaviani and Agosti (ALOA¹⁾) [1]

See B.3.

5.4.2 Second medium

The choice of the second medium is left to the discretion of the testing laboratory. If a commercial medium is used, the manufacturer's instructions shall be precisely followed regarding its preparation for use. **A1**

5.5 Solid culture medium: Tryptone soya yeast extract agar (TSYEA)

See Clause B.5.

5.6 Liquid culture medium: Tryptone soya yeast extract broth (TSYEB)

See Clause B.6.

5.7 Sheep blood agar

See Clause B.7.

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.

5.8 Carbohydrate utilization broth (rhamnose and xylose)

See Clause B.8.

5.9 Motility agar (optional)

See Clause B.9.

5.10 CAMP (Christie, Atkins, Munch-Petersen) medium and test strains

See Clause B.10.

5.11 Hydrogen peroxide solution

See Clause B.11.

5.12 Phosphate-buffered saline (PBS)

See Clause B.12.

6 Apparatus and glassware

Usual microbiological equipment (see ISO 7218) and, in particular, the following.

6.1 Apparatus for dry sterilization (oven) or wet sterilization (autoclave)

See ISO 7218.

6.2 Drying cabinet or incubator, capable of being maintained at between $25\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ and $50\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$.

6.3 Incubators, for maintaining the inoculated media, plates and tubes within the following temperature ranges:

- a) $25\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$;
- b) $30\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$; and
- c) $35\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ or $37\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$.

6.4 Water bath, capable of being maintained at $47\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$.

6.5 Loops, of platinum/iridium or nickel/chromium, approximately 3 mm in diameter, and *wires* of the same material, or *hockey-stick-shaped glass rods* or *single-use loops*.

6.6 pH-meter, capable of being read to the nearest 0,01 pH unit at $25\text{ }^{\circ}\text{C}$, enabling measurements to be made which are accurate to $\pm 0,1$ pH unit.

6.7 Test tubes or flasks, of appropriate capacity, for sterilization and storage of culture media and incubation of liquid media.

6.8 Measuring cylinders, of capacity 50 ml to 1 000 ml, for preparation of dilutions and complete media.

6.9 Total-delivery graduated pipettes, of nominal capacities 10 ml and 1 ml, graduated respectively in 0,5 ml and 0,1 ml divisions.

6.10 Petri dishes, of diameter 90 mm to 100 mm.

6.11 Jars, suitable for microaerobic incubation (optional).

6.12 Gas mixture (optional), of specified composition for microaerobic incubation:

5 % to 12 % CO_2 , 5 % to 15 % O_2 , and 75 % N_2 .

6.13 Equipment for the Henry illumination test (optional).

See Annex C.

6.14 Microscope, preferably with phase-contrast, and with slides and coverslips.

7 Sampling

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

Sampling is not part of the method specified in this part of ISO 11290. If there is no specific International Standard dealing with sampling of the product concerned, it is recommended that the parties concerned come to an agreement on this subject.

8 Preparation of test sample

Prepare the test sample in accordance with the specific International Standard appropriate to the product concerned. If there is no specific International Standard, it is recommended that the parties concerned come to an agreement on this subject.

9 Procedure

9.1 Test portion and initial suspension

See ISO 6887 and any specific International Standard appropriate to the product concerned.

For preparation of the initial suspension, use as dilution fluid the selective primary enrichment medium specified in 5.2.

In general, to prepare the initial suspension, add a test portion of x g or x ml to $9x$ ml or $9x$ g of the selective primary enrichment medium (5.2), to obtain a ratio of test portion to selective primary enrichment medium of 1/10 (mass to volume or volume to volume).

9.2 Primary enrichment

Incubate [6.3b)] the initial suspension, prepared in accordance with 9.1, at 30 °C for 24 h $\pm$ 2 h.

NOTE 3 A black coloration may develop during the incubation.

9.3 Secondary enrichment

9.3.1 After incubation of the initial suspension (primary enrichment) for 24 h $\pm$ 2 h (9.2), transfer 0,1 ml of the culture obtained in 9.2 (regardless of its colour) to a tube (6.7) containing 10 ml of secondary enrichment medium (Fraser broth) (5.3).

9.3.2 Incubate the inoculated medium (9.3.1) for 48 h $\pm$ 2 h at 35 °C or 37 °C.

NOTE 4 The temperature of the inoculated medium should be agreed upon between the parties concerned and recorded in the test report.

9.4 Plating out and identification

9.4.1 From the primary enrichment culture incubated for 24 h $\pm$ 3 h at 30 °C (9.2), take, by means of a loop or glass rod (6.5), a portion of the culture and inoculate the surface of the first selective plating medium, Agar *Listeria* according to Ottaviani and Agosti (5.4.1), so that well-separated colonies are obtained.

Proceed in the same way with the second selective plating-out medium (5.4.2). 

9.4.2 From the secondary enrichment medium incubated for 48 h $\pm$ 2 h at 35 °C or 37 °C (9.3.2), repeat the procedure described in 9.4.1 with the two selective plating-out media.

9.4.3 Invert the dishes obtained in 9.4.1 and 9.4.2 and place them in an incubator set at 37 °C for Agar *Listeria* according to Ottaviani and Agosti (5.4.1) and at the appropriate temperature for the second selective medium (5.4.2). If a commercial medium is used for the second selective medium, follow the manufacturer's instructions.

9.4.4 After incubation for 24 h $\pm$ 3 h (and for an additional 24 h $\pm$ 3 h if the growth is weak or if no colony is observed after 24 h incubation) for Agar *Listeria* according to Ottaviani and Agosti or for the appropriate time (second selective agar), examine the dishes (9.4.3) for the presence of colonies presumed to be *Listeria* spp. 