SLOVENSKI STANDARD
SIST EN ISO 11290-1:1997

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Mikrobiologija živil in krme - Horizontalna metoda za ugotavljanje prisotnosti in števila Listeria monocytogenes - 1. del: Metoda za ugotavljanje prisotnosti (ISO 11290-1:1996)

Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of Listeria monocytogenes - Part 1: Detection method (ISO 11290-1:1996)

Mikrobiologie von Lebensmitteln und Futtermitteln - Horizontales Verfahren für den Nachweis und die Zählung von Listeria monocytogenes - Teil 1: Nachweisverfahren (ISO 11290-1:1996)

Microbiologie des aliments - Méthode horizontale pour la recherche et le dénombrement de Listeria monocytogenes - Partie 1: Méthode de recherche (ISO 11290-1:1996)

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Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of Listeria monocytogenes - Part 1: Detection method (ISO 11290-1:1996)

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Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

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CEN

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

Central Secretariat: rue de Stassart,36 B-1050 Brussels

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of an identical text or by endorsement, at the latest by June 1997, and conflicting national standards shall be withdrawn at the latest by June 1997.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.

INTERNATIONAL STANDARD

ISO 11290-1

First edition
1996-12-15

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

Part 1: Detection method

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

Partie 1: Méthode de recherche

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Contents

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liason with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 11290-1 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 9, *Microbiology*.

ISO 11290 consists of the following parts, under the general title *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of* Listeria monocytogenes:

— *Part 1: Detection method*

— *Part 2: Enumeration method*

Annexes A and B form an integral part of this part of ISO 11290. Annex C is for information only.

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.

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

Part 1:
Detection method

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.

4.3 Plating out and identification

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

a) Oxford agar;

b) PALCAM agar.

Incubation at 30 °C, 35 °C or 37 °C and examination after 24 h and, if necessary, after 48 h to check for the presence of characteristic colonies which are presumed to be *L. monocytogenes*.

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

5.4.1 First medium: Oxford agar

See clause B.3.

5.4.2 Second medium: PALCAM agar

See clause B.4.

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.

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 °C ± 1 °C and 50 °C ± 1 °C.

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

a) 25 °C ± 1 °C;

b) 30 °C ± 1 °C; and

c) 35 °C ± 1 °C or 37 °C ± 1 °C.

6.4 Water bath, capable of being maintained at 47 °C ± 2 °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 °C, enabling measurements to be made which are accurate to ± 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.