INTERNATIONAL STANDARD

ISO 11290-2

Second edition
2017-05

Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. —

Part 2: Enumeration method

Microbiologie de la chaîne alimentaire — Méthode horizontale pour la recherche et le dénombrement de Listeria monocytogenes *et de* Listeria *spp.* —

Partie 2: Méthode de dénombrement

Reference number
ISO 11290-2:2017(E)

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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 liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

This document was prepared by the European Committee for Standardization (CEN) Technical Committee CEN/TC 275, *Food analysis — Horizontal methods*, in collaboration with ISO Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*, in accordance with the agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This second edition cancels and replaces the first edition (ISO 11290-2:1998), which has been technically revised. It also incorporates the amendment ISO 11290-2:1998/Amd.1:2004.

The main changes, compared to ISO 11290-2:1998, are the following.

— The enumeration of *Listeria monocytogenes* has been modified as listed below.

— Primary suspension with buffered peptone water, half-Fraser broth with or without supplements, and all appropriate diluents referred to in ISO 6887 (all parts).

— Resuscitation step deleted.

— Microscopic aspect, catalase and CAMP test for confirmation are optional.

— Inclusion of new performance characteristics.

— Moreover, enumeration of *Listeria* spp. has been included in the scope and the title changed accordingly.

A list of parts in the ISO 11290 series can be found on the ISO website.

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Introduction

The main changes, listed in the foreword, introduced in this document compared to ISO 11290-2:1998 are considered as major (see ISO 17468[28]). The technical changes were assessed and were considered to have no significant effect on the method performance characteristics or test results.

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, this horizontal method is intended to be applied as far as possible and deviations from this only be made for justified technical reasons.

When this document 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 document so that eventually the only remaining departures from this horizontal method will be those necessary for well-established technical reasons.

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Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. —

Part 2: Enumeration method

WARNING — In order to safeguard the health of laboratory personnel, it is essential that tests for detecting *L. monocytogenes* and *Listeria* spp. are only 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. Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety aspects, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices. In particular, it is strongly recommended that tests for detecting *L. monocytogenes* are undertaken in laboratories providing biosafety level 2 conditions. It is strongly recommended that female laboratory staff are made aware of the particular risk to the developing foetus presented by infection of the mother through exposure to *L. monocytogenes* and *Listeria* spp., and that pregnant personnel and persons with recognized underlying conditions or diseases that impair cell-mediated immunity do not manipulate cultures of *L. monocytogenes* and *Listeria* spp.

1 Scope

This document specifies a horizontal method for

— the enumeration of *L. monocytogenes*, and

— the enumeration of *Listeria* spp. (including *L. monocytogenes*).

This document is applicable to

— products intended for human consumption and for the feeding of animals, and

— environmental samples in the area of food production and food handling.

It is possible that certain additionally described *Listeria* species may not be enumerated or confirmed by this method.[3],[6],[9],[11]

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 6887 (all parts), *Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination*

ISO 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

ISO 11133, *Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media*

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ISO 11290-1, *Microbiology of the food chain — Horizontal method for the detection and enumeration of Listeria monocytogenes* and of *Listeria* spp. — Part 1: Detection method

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at http://www.iso.org/obp

3.1
Listeria monocytogenes
microorganisms which form typical colonies on solid selective media described and which display the morphological, physiological and biochemical characteristics described when the analysis is carried out in accordance with this document

3.2
enumeration of *Listeria monocytogenes*
determination of the number of colony-forming units (cfu) of *Listeria monocytogenes*, per gram, per millilitre, per square centimetre, or per sampling device when the analysis is carried out in accordance with this document

3.3
***Listeria* spp.**
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 document

3.4
enumeration of *Listeria* spp.
determination of the number of colony-forming units (cfu) of *Listeria* spp per gram, per millilitre, per square centimetre, or per sampling device, when the analysis is carried out in accordance with this document

4 Principle

4.1 General

Within the limits of this document, the enumeration of *L. monocytogenes* and of *Listeria* spp. requires five successive steps, as described in the flowchart in Annex A.

4.2 Initial suspension

Preparation of the initial suspension in an appropriate diluent according to the sample type.

4.3 Surface plating

Surface plating on Agar *Listeria* according to Ottaviani and Agosti[13],[14] of a specified quantity of the test sample for liquid products or of the initial suspension for other products and/or decimal dilutions if required.

4.4 Incubation

Incubation of the Petri dishes at 37 °C and examination after 24 h and after a further 24 h.

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