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Mikrobiologija v prehranski verigi - Horizontalna metoda za ugotavljanje prisotnosti in števila Listeria monocytogenes in Listeria spp. - 2. del: 1. del: Metoda za ugotavljanje prisotnosti (ISO/DIS 11290-1:2014)

Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and other Listeria spp. - Part 1: Detection method (ISO/DIS 11290-1:2014)

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Mikrobiologie von Lebensmitteln und Futtermitteln - Horizontales Verfahren für den Nachweis und die Zählung von Listeria monocytogenes - Teil-1: Nachweisverfahren (ISO/DIS 11290-1:2014)

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Microbiologie de la chaîne alimentaire - Méthode horizontale pour la recherche et le dénombrement de Listeria monocytogenes et Listeria spp. - Partie 1: Méthode de recherche (ISO/DIS 11290-1:2014)

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

Part 1: Detection method

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

Partie 1: Méthode de recherche

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ISO/CEN PARALLEL PROCESSING

This draft has been developed within the European Committee for Standardization (CEN), and processed under the **CEN lead** mode of collaboration as defined in the Vienna Agreement.

This draft is hereby submitted to the ISO member bodies and to the CEN member bodies for a parallel five month enquiry.

Should this draft be accepted, a final draft, established on the basis of comments received, will be submitted to a parallel two-month approval vote in ISO and formal vote in CEN.

To expedite distribution, this document is circulated as received from the committee secretariat. ISO Central Secretariat work of editing and text composition will be undertaken at publication stage.

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. 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.

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.

ISO 11290-1 was prepared by Technical Committee CEN/TC 275, *Food analysis - Horizontal methods*, and by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology* in collaboration.

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

— *Part 1: Detection method*

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— *Part 2: Enumeration method*

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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.

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

WARNING — In order to safeguard the health of laboratory personnel, it is strongly recommended that tests for detecting *L. monocytogenes and Listeria* spp. are undertaken in laboratories providing biosafety level 2 conditions, under the control of a skilled microbiologist, and that great care is taken in the disposal of all contaminated materials. In particular, 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. National legislation may involve more specific demands. Only *L. monocytogenes* and *L. ivanovii* can give rise to listeriosis.

1 Scope

This part of ISO 11290 specifies a horizontal method for

— the detection of *L. monocytogenes*;

— the detection of *Listeria* spp.

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This part of ISO 11290 is applicable to:

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

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

NOTE Certain new *Listeria* species may not be detected or confirmed by this method (see references [4, 9, 11, 13]).

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 6887, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination (all parts).*

ISO 7218*, Microbiology of food and animal feeding stuffs — General rules 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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3 Terms and definitions

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

3.1
Listeria monocytogenes
microorganism 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 *Listeria monocytogenes* (3.1), in a given mass or volume of product or a specified surface, when tests are carried out in accordance with this part of ISO 11290.

3.3
***Listeria* spp.**
microorganism 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.

Certain new *Listeria* species may not be detected or confirmed by this method (see References [4, 9, 11, 13]).

3.4
detection of *Listeria* spp.
determination of the presence or absence of *Listeria* spp.(3.3) , in a given mass or volume of product or a specified surface, when tests are carried out in accordance with this part of ISO 11290.

4 Principle

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.

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

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 half the concentrations of acriflavine and nalidixic acid (half-Fraser broth), which is also used as a dilution fluid for the test portion (9.1).

Incubation of the initial suspension at 30 °C ± 1 °C for 24 h ± 2 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 37 °C ± 1 °C for 24 h ± 2 h.

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4.3 Plating out and identification

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

- Agar *Listeria* according to Ottaviani and Agosti (see Reference [1] and B.3);
- any other solid selective medium at the choice of the laboratory complementary to Agar *Listeria* according to Ottaviani and Agosti, such as for example Oxford, PALCAM, or another medium using a different substrate than the one used in *Listeria* agar according to Ottaviani and Agosti (B.4). See Annex E for some information about media.

Incubation of the Agar *Listeria* according to Ottaviani and Agosti at 37 °C ± 1 °C and examination after 24 h ± 2 h, and after a further 24 h ± 2 h, to check for the presence of characteristic colonies which are presumed to be *L. monocytogenes* or *Listeria* spp. Incubation of the second selective medium at the appropriate temperature and examination after the appropriate time.

4.4 Confirmation

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

5 Culture media and reagents

For current laboratory practice, see ISO 11133.

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Composition of culture media and reagents and their preparation are described in Annex B.

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6 Apparatus and glassware

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

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

See ISO 7218.

6.2 Incubators, capable of operating at 30 °C ± 1 °C, 37 °C ± 1 °C and at 25 °C ± 1 °C.

6.3 Waterbath, capable of operating at 47 °C to 50 °C.

6.4 Drying cabinet or incubator, capable of being maintained between 25 °C and 50 °C.

6.5 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.

6.6 Loops, of platinum/iridium or nickel/chromium, approximately 3 mm in diameter or 10 µl, and **wires** of the same material, or single-use loops.

6.7 Graduated pipettes or automatic pipettes, of nominal capacities 10 ml and 1 ml, graduated respectively in 0,5 ml and 0,1 ml divisions.

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

6.9 Petri dishes, of diameter 90 mm.

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7 Sampling

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. For environmental samples, refer to ISO18593 [12].

It is important that the laboratory receives a sample which is truly representative and has not been damaged or changed during transport or storage (see ISO 7218).

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 B.1. (half-Fraser broth).

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

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9.2 Primary enrichment

Incubate the primary enrichment medium (half-Fraser broth), prepared in accordance with 9.1, at 30 °C ± 1 °C for 24 h ± 2 h.

NOTE 1 A black coloration may develop during the incubation.

NOTE 2 A minimum of 24 h incubation is recommended, in particular in the case of stress or low contamination levels of *Listeria* spp. in the samples.

9.3 Secondary enrichment

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

9.3.2 Incubate the inoculated medium (9.3.1) for 24 h ± 2 h at 37 °C ± 1 °C.

NOTE 1 In the case of *Listeria* spp. detection, additional 24 h incubation may allow recovery of more species.

NOTE 2 Half-Fraser broth may be refrigerated before transfer to Fraser broth during a maximum of 72 h, if (i) a validation study for such storage of the commercially available broth is available or (ii) the laboratory has performed a verification study of the method using such storage, according to ISO 16140-3 [1].

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9.4 Plating out and identification

9.4.1 From the primary enrichment culture incubated for 24 h ± 2 h at 30 °C, inoculate, by means of a loop (6.6), the surface of the first selective plating medium, Agar *Listeria* according to Ottaviani and Agosti (B.3), to obtain well-separated colonies.

Proceed in the same way with the second selective plating-out medium of choice (B.4).

NOTE Half-Fraser broth and Fraser broth may be refrigerated before isolation on selective agar during a maximum of 72 h, if (i) a validation study for such storage of the commercially available broth is available or (ii) the laboratory has performed a verification study of the method using such storage, according to ISO 16140-3 [1].

9.4.2 From the secondary enrichment medium incubated for 24 h ± 2 h at 37 °C ± 1 °C (9.3.2), repeat the procedure described in 9.4.1 with the two selective plating-out media.

9.4.3 Invert the Petri dishes obtained in 9.4.1 and 9.4.2 and place them in an incubator set at 37 °C ± 1 °C for Agar *Listeria* according to Ottaviani and Agosti (B.3) and for the second selective medium (B.4), follow the manufacturer's instructions.

9.4.4 After incubation for 24 h ± 2 h and for an additional 24 h ± 2 h 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 *L. monocytogenes* and/or *Listeria* spp. Incubated plates can be refrigerated for a maximum of 2 days before reading.

9.4.4.1 Agar *Listeria* according to Ottaviani and Agosti

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Consider as *L. monocytogenes* the blue-green colonies surrounded by an opaque halo (typical colonies).Colonies of *L. ivanovii* are also blue-green and surrounded by an opaque halo.

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

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NOTE 2 Some rare *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 [2]). No *L.* monocytogenes strains have been described as PIPLC negative.

Consider as *Listeria* spp. blue-green colonies with or without opaque halo.

NOTE 3 Some new *Listeria* species (such as *L. rocourtiae*), which have been recently characterized, may not grow on Agar *Listeria* according to Ottaviani and Agosti (see Reference [13]).

NOTE 4 Some organisms other than *Listeria* spp. may produce blue colonies on this medium. See Annex C.

9.4.4.2 Second selective medium

After the appropriate time, examine the plates for the presence of colonies which are considered to be presumptive *Listeria* spp. or *L. monocytogenes*, based on their characteristics for the type of medium used.

9.5 Confirmation of *Listeria monocytogenes* and/or *Listeria* spp.

9.5.1 Selection of colonies for confirmation

9.5.1.1 For confirmation of presumptive *L. monocytogenes*, take from each plate of each selective medium (see 9.4.4.1 and 9.4.4.2) at least one colony presumed to be *L. monocytogenes* and a further four colonies if the first is negative. One confirmed isolate per sample is sufficient.

9.5.1.2 For confirmation of presumptive *Listeria* spp., take from each plate of each selective medium (see 9.4.4.1 and 9.4.4.2) at least one colony presumed to be *Listeria* spp. and a further four colonies if the first is negative. One confirmed isolate per sample is sufficient.

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