SLOVENSKI STANDARD
oSIST prEN ISO 10272-2:2015

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Mikrobiologija živil in krme - Horizontalna metoda za ugotavljanje prisotnosti in števila Campylobacter spp. - 2. del: Tehnika štetja kolonij (ISO/DIS 10272-2:2015)

Microbiology of the food chain - Horizontal method for detection and enumeration of Campylobacter spp. - Part 2: Colony-count technique (ISO/DIS 10272-2:2015)

Mikrobiologie der Lebensmittelkette - Horizontales Verfahren zum Nachweis und zur Zählung von Campylobacter - Teil 2: Koloniezählverfahren (ISO/DIS 10272-2:2015)

Microbiologie de la chaîne alimentaire - Méthode horizontale pour la recherche et le dénombrement de Campylobacter spp. - Partie 2 : Technique par comptage des colonies (ISO/DIS 10272-2:2015)

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07.100.30	Mikrobiologija živil	Food microbiology

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Microbiology of food and animal feed — Horizontal method for detection and enumeration of Campylobacter —

Part 2:
Colony-count technique

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

Partie 2: Technique par comptage des colonies

ICS: 07.100.30

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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Contents

Page

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 10272-2 was prepared by the European Committee for Standardization (CEN), in collaboration with Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

This second edition cancels and replaces the first edition (ISO/TS 10272-2:2006), which has been technically revised and performance characteristics have been added.

ISO 10272 consists of the following parts, under the general title *Microbiology of the food chain— Horizontal method for detection and enumeration of* Campylobacter:

— *Part 1: Detection method*

— *Part 2: Colony-count technique*

Introduction

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

When this Technical Specification 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 this in the case of particular products. The harmonization of test methods cannot be immediate and, for certain group 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 International Standard, 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 feed — Horizontal method for detection and enumeration *of* Campylobacter — Part 2: Colony-count technique

1 Scope

This part of ISO 10272 describes a horizontal method for the enumeration of *Campylobacter*.

It is applicable to products intended for human consumption or for the feeding of animals, and to environmental samples in the area of food production and food handling, subject to the limitations stated in the Introduction.

2 Normative references

The following referenced documents are indispensable for the application 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 food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination.*

ISO 7218/Amd1 *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.

3 Terms and definitions

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

3.1
Campylobacter
microorganisms forming characteristic colonies on solid selective media when incubated in a microaerobic atmosphere at 41,5 °C, and which possess the characteristic morphology and motility and biochemical and growth properties described when the tests are conducted in accordance with this part of ISO 10272.

NOTE The most frequently encountered species are *Campylobacter jejuni* and *Campylobacter coli*. Other species have, however, been described (*Campylobacter lari*, *Campylobacter upsaliensis* and others).

3.2
enumeration of *Campylobacter*
determination of the number of colony-forming units (cfu) of *Campylobacter* (3.1) found per millilitre, per gram or per cm^2 of test sample when the test is conducted in accordance with this part of ISO 10272.

4 Principle

4.1 Preparation of dilutions

For the preparation of decimal dilutions from the test sample, see ISO 6887.

4.2 Enumeration

The solid selective medium, modified charcoal cefoperazone deoxycholate agar (mCCD agar), is inoculated with a specified quantity of the test sample if the product is liquid, or of the initial suspension in the case of other products.

Other plates are prepared under the same conditions, using decimal dilutions of the test sample or of the initial suspension.

The plates are incubated at 41,5 °C in a microaerobic atmosphere and examined after 44 h ± 4 h to record the number of colonies presumed because of their characteristics to be *Campylobacter*.

4.3 Confirmation

The colonies presumed to be *Campylobacter* are examined for morphology and motility using a microscope and sub-cultured on a non-selective blood agar, and then confirmed by detection of oxidase and an aerobic growth test at 25°C. Optionally, the *Campylobacter* species are identified by specific biochemical tests and/or molecular methods.

The number of colony-forming units (cfu) *Campylobacter* per ml,per g or per cm^2 of the test sample is calculated from the number of confirmed typical colonies per plate.

5 Culture media and reagents

5.1 General

For current laboratory practice, see ISO11133.

NOTE Because of the large number of culture media and reagents and for the clarity of the text, their compositions and preparations are given in Annex B.

5.2 Diluent

See ISO 6887.

5.3 Selective plating medium: Modified charcoal cefoperazone deoxycholate agar (mCCD agar)

See B.1.

5.4 Confirmation and identification media and reagents

5.4.1 Blood agar

See B.2.

5.4.2 Reagent for the detection of oxidase

See B.3.

5.4.3 Hydrogen peroxide solution, 3 % (volume fraction)

5.4.4 Reagents for the detection of hydrolysis of hippurate

See B.4.

5.4.5 Indoxyl acetate discs

See B.5.

6 Apparatus and glassware

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

6.1 Incubators, capable of operating at 25 °C ± 1 °C, 37 °C ± 1 °C and 41,5 °C ± 1 °C.

6.2 Water bath, capable of operating at 37 °C ± 1 °C

6.3 Sterile loops, of platinum/iridium, nickel/chromium or plastic, approximately 3 mm in diameter, and **wires** of the same material, or a glass or plastic **rod**. A nickel/chromium loop is not suitable for use in the oxidase test (see 9.4.3).

6.4 Microscope, preferably with phase contrast (for observing the characteristic morphology and motility of *Campylobacter*).

6.5 Appropriate apparatus for achieving a microaerobic atmosphere with oxygen content of 5 % ± 2 %, carbon dioxide 10 % ± 3 %, optional hydrogen ≤10 %, with the balance nitrogen. The appropriate microaerobic atmosphere can be obtained using gastight jars and gas-generating kits, following precisely the manufacturer's instructions. Alternatively, the jar or incubator may be filled with an appropriate gas mixture prior to incubation.

7 Sampling

It is important that the laboratory receives 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 10272. See the specific International Standard dealing with the product concerned. 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.

Since *Campylobacter* is very sensitive to freezing but survives best at low temperatures, samples to be tested should not be frozen, but stored at 3 °C ± 2 °C and subjected to analysis as rapidly as possible. Also take care to prevent the samples from drying.

8 Preparation of test sample

Prepare the test sample in accordance with the specific International Standard dealing with 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 **(see diagram in Annex A)**

9.1 Test portion, initial suspension and dilutions

See ISO 6887 and the specific International Standard dealing with the product concerned.

Prepare a single decimal dilution series from the test sample if the product is liquid, or from the initial suspension in the case of other products.