INTERNATIONAL STANDARD

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Microbiology of food and animal feeding stuffs — Horizontal method for detection and enumeration of *Campylobacter* spp. —

Part 1: Detection method

Microbiologie des aliments — Méthode horizontale pour la recherche et le dénombrement de Campylobacter *spp. —*

Partie 1: Méthode de recherche

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ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

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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 10272-1 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

This first edition of ISO 10272-1, together with ISO/TS 10272-2:2006, cancels and replaces ISO 10272:1995, which has been technically revised.

ISO 10272 consists of the following parts, under the general title *Microbiology of food and animal feeding stuffs — Horizontal method for detection and enumeration of* Campylobacter *spp.*:

— *Part 1: Detection method*

— *Part 2: Colony-count technique* (Technical Specification)

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 International Standard 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 feeding stuffs — Horizontal method for detection and enumeration of *Campylobacter* spp. —

Part 1: Detection method

1 Scope

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

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, *Microbiology of food and animal feeding stuffs — General rules for microbiological examinations*

ISO 8261, *Milk and milk products — General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examinations*

ISO/TS 11133-1, *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory*

ISO/TS 11133-2:2003, *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 2: Practical guidelines on 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 microaerobically at 41,5 °C but not at 25 °C, and which possess the characteristic 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 some others).

3.2
detection of *Campylobacter*
determination of the presence or absence of these microorganisms in a defined quantity of product, when the test is conducted in accordance with this part of ISO 10272

4 Principle

4.1 General

In general, the detection of *Campylobacter* requires the following stages (see Annex A for a diagram of the procedure).

4.2 Enrichment in selective liquid medium

The test portion is inoculated into the liquid enrichment medium (Bolton broth) and homogenized.

It is incubated in a microaerobic atmosphere at 37 °C for 4 h to 6 h and then at 41,5 °C for 44 h ± 4 h.

4.3 Isolation and selection for confirmation

From the cultures obtained in 4.2, two selective solid media are inoculated:

— modified charcoal cefoperazone deoxycholate agar (mCCD agar);

— any other solid selective medium based on a principle different from that of mCCD agar.

They are then incubated at 41,5 °C in a microaerobic atmosphere and inspected after 44 h ± 4 h to detect the presence of colonies presumed because of their characteristics to be *Campylobacter*.

4.4 Confirmation

The colonies presumed to be *Campylobacter* are subcultured on the non-selective Columbia blood agar, then confirmed by means of microscopic examination and appropriate biochemical and growth tests. Optionally, the *Campylobacter* species are identified by specific biochemical tests and antibiotic sensitivity tests.

5 Culture media and reagents

5.1 General

For current laboratory practice, see ISO 7218, ISO/TS 11133-1 and ISO/TS 11133-2.

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 Liquid enrichment medium: Bolton broth

See B.1.

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

See B.2.

5.4 Confirmation and identification media and reagents

5.4.1 Columbia blood agar

See B.3.

5.4.2 Brucella broth

See B.4.

5.4.3 Reagent for the detection of oxidase

See B.5.

5.4.4 Hydrogen peroxide solution, 3 % (volume fraction)

5.4.5 Reagents for the detection of hydrolysis of hippurate

See B.6.

5.4.6 Mueller Hinton blood agar

See B.7.

5.4.7 Nalidixic acid (30 µg) and cephalothin (30 µg) discs

5.4.8 Indoxyl acetate discs

See B.8.

6 Apparatus and glassware

Usual microbiological laboratory 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 **Oven, laminar flow cabinet or incubator**, capable of operating between 37 °C and 55 °C.

6.3 **Incubator**, capable of operating at 41,5 °C ± 1 °C.

6.4 **Water baths**, capable of operating at 25 °C ± 1 °C and 37 °C ± 1 °C, or incubators capable of operating at 25 °C ± 1 °C and 37 °C ± 1 °C.

6.5 **Water bath**, capable of operating between 47 °C and 50 °C.

6.6 **pH-meter**, accurate to within 0,1 pH units at 25 °C.

6.7 Containers, in particular culture tubes of dimensions 18 mm × 180 mm and 9 mm × 180 mm, haemolysis tubes of dimensions 13 mm × 75 mm, bottles with non-toxic metal closures and/or flasks of appropriate capacity with appropriate covers.

6.8 Petri dishes, in glass or plastic, with diameters 90 mm to 100 mm.

6.9 Total-delivery graduated pipettes, with a wide opening, and a nominal capacity of 1 ml and 10 ml, graduated in 0,1 ml divisions, and **Pasteur pipettes**.

6.10 Rubber teats, or any other safety system capable of being adapted to the graduated pipettes.

6.11 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 9.4.6).

6.12 Forceps, fine, round-ended, of stainless steel.

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

6.14 Apparatus suitable for achieving a microaerobic atmosphere with oxygen content of 5 % ± 2 %, carbon dioxide 10 % ± 3 %, optional hydrogen ⩽ 10 %, with the balance nitrogen. Appropriate gastight containers are used to hold Petri dishes and/or flasks or bottles of about 350 ml capacity used for the enrichment broth, e.g. bacteriological anaerobic jars.

NOTE 1 The appropriate microaerobic atmosphere can be obtained using commercially available gas-generating kits, following precisely the manufacturer's instructions, particularly those relating to the volume of the jar and the capacity of the gas-generating kit. Alternatively, the jar may be filled with an appropriate gas mixture prior to incubation.

NOTE 2 As an alternative to incubation in a microaerobic atmosphere, the enrichment broth can be incubated in screw-capped bottles or flasks filled with enrichment broth, leaving a headspace of less than 2 cm, and tightly closing the caps.

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 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* spp. are very sensitive to freezing but survive best at low temperatures, it is recommended that 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

9.1.1 See the suitable part of ISO 6887, or ISO 8261.

9.1.2 In general, for preparing the initial suspension, introduce a quantity x of the test portion (mass or volume) into nine times its volume of the enrichment medium Bolton broth (5.2), so as to obtain a test portion/enrichment medium ratio of 1:10 (mass/volume or volume/volume), and homogenize.

9.2 Enrichment

Incubate the initial suspension (9.1.2) in a microaerobic atmosphere (6.14) at 37 °C for 4 h to 6 h, then at 41,5 °C for 44 h ± 4 h.

9.3 Isolation

9.3.1 Using the culture obtained in the enrichment medium (9.2), inoculate with a sterile loop (6.11) the surface of the first selective isolation medium, mCCD agar (5.3).

Proceed in the same manner with the second *Campylobacter* selective isolation medium chosen.

NOTE It is preferable to take a second isolation medium that is based on a principle different from mCCD agar. Examples of isolation media to be used are Skirrow agar, Karmali agar and Preston agar (see Bibliography).

9.3.2 Incubate the plates (9.3.1) at 41,5 °C in a microaerobic atmosphere (6.14).

9.3.3 After 44 h ± 4 h of incubation, examine the plates for typical and/or suspect colonies of *Campylobacter.*

The typical colonies are greyish on mCCD agar, often with a metallic sheen, and are flat and moist, with a tendency to spread. Colonies spread less on drier agar surfaces. Other forms of colonies may occur.

9.4 Confirmation of *Campylobacter* species

9.4.1 General

As the bacteria rapidly deteriorate in air, follow the procedure described in 9.4.2 to 9.4.6 without any delay.

9.4.2 Selection of colonies for confirmation

9.4.2.1 For confirmation, take from each plate of each selective medium (9.3.1) at least one colony considered to be typical or suspected as being *Campylobacter* and a further four colonies if the first is negative.

9.4.2.2 Streak each of the selected colonies onto a Columbia blood agar plate (5.4.1) in order to allow the development of well-isolated colonies. Incubate the plates in a microaerobic atmosphere at 41,5 °C for 24 h to 48 h. Use the pure cultures for examination of morphology, motility, microaerobic growth at 25 °C, aerobic growth at 41,5 °C and the presence of oxidase.

9.4.3 Examination of morphology and motility

9.4.3.1 Suspend one colony from the Columbia blood agar plate (9.4.2.2) in 1 ml of Brucella broth (5.4.2) and examine for morphology and motility using a microscope (6.13).

9.4.3.2 Retain for further examination all cultures (9.4.2.2) in which curved bacilli with a spiralling “corkscrew” motility are found (9.4.3.1).