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

Part 1: Detection method

Microbiologie de la chaîne alimentaire — Méthode horizontale pour la recherche et le dénombrement de Campylobacter *spp. — Partie 1: Méthode de recherche*

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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 10272-1:2006), which has been technically revised with the following main changes:

— samples from the primary production stage have been added to the scope;

— the detection method was extended to include the option of a second enrichment broth (Preston broth), primarily to overcome problems with background flora resistant to third generation ß-lactams (like cefoperazone in Bolton broth);

— the detection method was extended to include the option of direct plating on mCCDA;

— the note on the use of closed containers with reduced headspace as an alternative to incubation in a microaerobic atmosphere has been deleted;

— the confirmation tests on study of microaerobic growth at 25 °C and aerobic growth at 41,5 °C were replaced by the study of aerobic growth at 25 °C;

— performance testing for the quality assurance of the culture media has been added to Annex B;

— performance characteristics have been added to Annex C.

A list of all parts in the ISO 10272 series can be found on the ISO website.

Introduction

The main changes, listed in the foreword, introduced in this document compared to ISO 10272-1:2006 are considered as minor (see ISO 17468).

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

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

Part 1: Detection method

WARNING — In order to safeguard the health of laboratory personnel, it is essential that tests for detecting *Campylobacter* 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.

1 Scope

This document specifies a horizontal method for the detection by enrichment or direct plating of *Campylobacter* spp. It is applicable to

— products intended for human consumption,

— products intended for animal feeding,

— environmental samples in the area of food and feed production, handling, and

— samples from the primary production stage such as animal faeces, dust, and swabs.

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*

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
Campylobacter
microorganism forming characteristic colonies on solid selective media when incubated in a microaerobic atmosphere at 41,5 °C, and which possesses the characteristic morphology and motility and biochemical and growth properties described when the tests are conducted in accordance with this document

Note 1 to entry: This document targets the thermotolerant *Campylobacter* species relevant to human health. The most frequently encountered and relevant to human health thermotolerant species are *Campylobacter jejuni* and *Campylobacter coli*. However, other species have been described *(Campylobacter lari*, *Campylobacter upsaliensis* and others).

3.2
detection of *Campylobacter*
determination of the presence or absence of *Campylobacter* (3.1) in a defined quantity of product, when the test is conducted in accordance with this document

4 Principle

4.1 General

The detection of *Campylobacter* requires three successive stages as specified in Annex A.

Depending on the type of sample and the purpose of the test, three different detection procedures can be used:

— **detection procedure A**: Detection of *Campylobacter* by enrichment, in samples with low numbers of campylobacters and low level of background microflora and/or with stressed campylobacters;

— **detection procedure B:** Detection of *Campylobacter* by enrichment, in samples with low numbers of campylobacters and high level of background microflora;

— **detection procedure C**: Detection of *Campylobacter* by direct plating, in samples with high numbers of campylobacters.

4.2 Enrichment in selective liquid medium

4.2.1 Detection procedure A

The test portion is added to the liquid enrichment medium (Bolton broth).

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.

4.2.2 Detection procedure B

The test portion is added to the liquid enrichment medium (Preston broth).

It is incubated in a microaerobic atmosphere at 41,5 °C for 24 h.

4.2.3 Detection procedure C

Enrichment technique is not used.

4.3 Isolation on selective solid medium

4.3.1 Detection procedure A

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

— modified Charcoal Cefoperozone Deoxycholate agar (mCCD agar);

— any other solid selective *Campylobacter* medium using different selective principles from those in mCCD agar.

4.3.2 Detection procedure B

From the enrichment culture obtained in 4.2, the selective mCCD agar is inoculated.

4.3.3 Detection procedure C

The test portion is plated directly or after suspending in an appropriate amount of liquid onto the selective mCCD agar.

4.3.4 Detection procedure A, B and C

The selective solid media are incubated at 41,5 °C in a microaerobic atmosphere and examined after 44 h to detect the presence of suspect *Campylobacter* colonies.

4.4 Confirmation

The suspect *Campylobacter* colonies 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 activity and an aerobic growth test at 25 °C. Optionally, the *Campylobacter* species are identified by specific biochemical tests and/or molecular methods.

5 Culture media and reagents

For current laboratory practice, see ISO 7218 and ISO 11133.

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

For performance testing of culture media, see Annex B.

6 Equipment and consumables

Disposable equipment is an acceptable alternative to reusable glassware if it has suitable specifications.

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 10 µl volume and of 1 µl volume, and **inoculation needle** or **wire**.

A nickel/chromium loop is not suitable for use in the oxidase test (see 9.5.5).

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

6.5 Apparatus suitable 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.

6.6 Sterile Petri dishes, with a diameter of approximately 90 mm, preferably with vents to facilitate microaerobic incubation.

6.7 Refrigerators, capable of operating at 3 °C ± 2 °C and at 5 °C ± 3 °C.

7 Sampling

Sampling is not part of the method specified in this document. See the specific International Standard dealing with the product concerned. If there is no specific International Standard dealing with the sampling of the product concerned, it is recommended that the parties concerned come to an agreement on this subject.

A recommended sampling method is given in ISO/TS 17728 for food and animal feed, in ISO 13307 for sampling at the primary production stage, in ISO 17604 for sampling of carcasses, and in ISO 18593 for sampling of surfaces.

It is important that the laboratory receives a sample that is representative and the sample should not have been damaged or changed during transport or storage.

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 (6.7) 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 from the laboratory sample in accordance with the specific International Standard dealing with the product concerned: see ISO 6887 (all parts). 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 General

Depending on the type of sample and the purpose of the test, one or more of three different detection procedures is/are used:

— **detection procedure A:** Detection of *Campylobacter* by enrichment, in samples with low numbers of campylobacters and low level of background microflora and/or with stressed campylobacters, e.g. cooked or frozen products;

— **detection procedure B:** Detection of *Campylobacter* by enrichment, in samples with low numbers of campylobacters and high level of background microflora, e.g. raw meats (including poultry) or raw milk;

— **detection procedure C:** Detection of *Campylobacter* by direct plating, in samples with high numbers of campylobacters, e.g. faeces, poultry caecal contents or raw poultry meat. This can be used in combination with ISO 10272-2 in order to count numbers of *Campylobacter* per g, per ml, or per cm^2 in the test material.

If little information is available concerning the best method for the particular type of sample to be tested, then use detection procedure C, in parallel with detection procedure(s) A and/or B.

In general, detection procedure B is useful for products (including cooked or frozen) that contain significant numbers of microflora resistant to third generation ß-lactams like cefoperazone. Cefoperazone is used in Bolton broth (detection procedure A) as well as in mCCD agar. Preston broth (detection procedure B) uses different selective principles and is therefore more suitable to suppress this type of resistant microflora.

9.2 Test portion and initial suspension

9.2.1 General

For preparation of the initial suspension, in the general case, use as diluent the enrichment medium specified in 9.2.2 or 9.2.3. Pre-warm the enrichment medium to room temperature before use.

In general, an amount of test portion (mass or volume) is mixed with a quantity of enrichment medium (mass or volume) to yield a tenfold dilution. However, for some types of samples (e.g. boot socks, swabs), it may be necessary to use another ratio.

This document has been validated for test portions of 10 g (or ml), except for the caecal samples. A smaller size of test portion may be used, without the need for additional validation/verification, providing that the same ratio between enrichment broth and test portion is maintained. A larger test portion than that initially validated may be used, if a validation/verification study has shown that there are no adverse effects on the detection of *Campylobacter*.

NOTE Validation can be conducted in accordance with the appropriate documents of ISO 16140 (all parts). Verification for pooling samples can be conducted in accordance with the protocol described in ISO 6887-1:2017, Annex D (verification protocol for pooling samples for qualitative tests).

9.2.2 Detection procedure A

In general, for preparing the initial suspension, combine a quantity of 10 g or 10 ml of the test portion with 90 ml of the enrichment medium Bolton broth (B.2), so as to obtain a 1 in 10 dilution, and homogenize (see ISO 7218).

9.2.3 Detection procedure B

In general, for preparing the initial suspension, combine a quantity of 10 g or 10 ml of the test portion with 90 ml of the enrichment medium Preston broth (B.3), so as to obtain a 1 in 10 dilution, and homogenize (see ISO 7218).

9.2.4 Detection procedure C

9.2.4.1 For caecal or faecal samples, use a loop (6.3) or a sterile swab to bring some of the well-mixed sample material onto the first half of a mCCD agar plate (B.4). Use another loop to streak out on the second half of the plate.

9.2.4.2 For all other samples, add an appropriate amount of liquid (e.g. peptone salt solution or Preston broth), for example, 1 in 2 (volume fraction), mix well, and either streak the plate using a loop (6.3), or dispense a suitable volume and spread it over the mCCD agar plate (B.4).

NOTE Using a second plating medium (B.5) with selective agents different from those in mCCD agar could improve *Campylobacter* detection, especially in the presence of background flora resistant to 3rd generation ß-lactams like cefoperazone.

9.3 Enrichment

9.3.1 Detection procedure A

Incubate the initial suspension (9.2.2) in a microaerobic atmosphere (6.5) at 37 °C (6.1) for 4 h to 6 h, then at 41,5 °C (6.1) for 44 h ± 4 h.

9.3.2 Detection procedure B

Incubate the initial suspension (9.2.3) in a microaerobic atmosphere (6.5) at 41,5 °C (6.1) for 24 h ± 2 h.

9.4 Isolation

9.4.1 Detection procedure A

Using the culture obtained in the enrichment medium (9.3.1), inoculate with a sterile 10 µl loop (6.3) the surface of the first selective isolation medium, mCCD agar (B.4).

Proceed in the same manner with the second *Campylobacter* selective isolation medium chosen (B.5).

9.4.2 Detection procedure B

Using the culture obtained in the enrichment medium (9.3.2), inoculate with a sterile 10 µl loop (6.3) the surface of the isolation medium, mCCD agar (B.4).

9.4.3 Detection procedures A, B and C

Incubate the plates (9.2.4, 9.4.1 and 9.4.2) at 41,5 °C (6.1) in a microaerobic atmosphere (6.5).

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

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

NOTE The recognition of colonies of *Campylobacter* is to a large extent a matter of experience and their appearance can vary somewhat, not only from strain to strain, but also from batch to batch of the selective culture medium used.

9.5 Confirmation of *Campylobacter*

9.5.1 General

As *Campylobacter* rapidly loses culturability in air, follow the procedure described in 9.5.2 to 9.5.5 without delay.

For a clear distinction between positive and negative confirmation reactions, it is helpful to verify this with well-characterized positive and negative control strains. Examples of suitable control strains are *Campylobacter jejuni* WDCM 00005 (positive control)[17] and *Escherichia coli* WDCM 00013 (negative control).

As an alternative, or in addition, to the confirmation and identification tests described in this document, other tests (PCR tests, serological methods, matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOF-MS) analysis, etc.) can be used, providing the suitability of the alternative procedure is verified (see ISO 7218).