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

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

Reference number
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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 first edition cancels and replaces ISO/TS 10272-2:2006, which has been technically revised with the following main changes:

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

— serial dilutions are plated in single instead of in duplicate, to be in line with ISO 7218;

— 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.

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Introduction

The main changes, listed in the foreword, introduced in this document compared to ISO/TS 10272-2:2006 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.

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

Part 2: Colony-count technique

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

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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 are *Campylobacter jejuni* and *Campylobacter coli*. However, other species have 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 gram, per millilitre, per square centimetre or per sampling device when the test is conducted in accordance with this document

4 Principle

4.1 General

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

4.2 Preparation of dilutions

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

4.3 Enumeration

The solid selective medium modified Charcoal Cefoperozone Deoxycholate agar (mCCD agar) is inoculated with a specified quantity of the test portion 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 portion or of the initial suspension.

The plates are incubated at 41,5 °C in a microaerobic atmosphere and examined after 44 h to record the number 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.

The number of colony-forming units (cfu) of *Campylobacter* per unit of the test portion is calculated from the number of confirmed typical colonies per plate.

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.

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