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

Part 2:

Colony-count technique

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

ISO/TS 10272-2:2006
Partie 2: Technique par comptage des colonies

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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.

In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of normative document:

- an ISO Publicly Available Specification (ISO/PAS) represents an agreement between technical experts in an ISO working group and is accepted for publication if it is approved by more than 50 % of the members of the parent committee casting a vote;
- an ISO Technical Specification (ISO/TS) represents an agreement between the members of a technical committee and is accepted for publication if it is approved by 2/3 of the members of the committee casting a vote.

An ISO/PAS or ISO/TS is reviewed after three years in order to decide whether it will be confirmed for a further three years, revised to become an International Standard, or withdrawn. If the ISO/PAS or ISO/TS is confirmed, it is reviewed again after a further three years, at which time it must either be transformed into an International Standard or be withdrawn.

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/TS 10272-2 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

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

ISO/TS 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 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.

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

Part 2: Colony-count technique

1 Scope

This Technical Specification describes a horizontal method for the enumeration 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.

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2 Normative references (standards.iteh.ai)

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 micro-aerobically 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 Technical Specification

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
count of *Campylobacter*
number of *Campylobacter* found per millilitre or per gram of test sample when the test is conducted in accordance with this Technical Specification

4 Principle

4.1 Preparation of dilutions

For the preparation of decimal dilutions from the test sample, see ISO 6887 and ISO 8261.

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 micro-aerobic atmosphere for 40 h to 48 h.

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

The number of *Campylobacter* per millilitre or per gram of the test sample is calculated from the number of confirmed typical colonies per plate.

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5 Culture media and reagents

5.1 General

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

5.2 Diluent

See ISO 6887.

5.3 Modified charcoal cefoperazone desoxycholate agar (mCCD agar)

5.3.1 Basic medium

5.3.1.1 Composition

Meat extract	10,0 g
Enzymatic digest of animal tissues	10,0 g
Sodium chloride	5,0 g
Charcoal	4,0 g
Enzymatic digest of casein	3,0 g
Sodium desoxycholate	1,0 g
Iron(II) sulfate	0,25 g
Sodium pyruvate	0,25 g
Agar	8,0 g to 18,0 g ^a
Water	1 000 ml

^a Depending on the gel strength of the agar.

5.3.1.2 Preparation

Dissolve the basic components or the dehydrated complete basic medium in the water, by bringing to the boil. Adjust the pH, if necessary, so that after sterilization it is $7,4 \pm 0,2$ at 25 °C. Dispense the basic medium into flasks of suitable capacity. Sterilize in the autoclave (6.1) set at 121 °C for 15 min.

5.3.2 Antibiotic solution

5.3.2.1 Composition

Cefoperazone	0,032 g
Amphotericin B	0,01 g
Water	5 ml

5.3.2.2 Preparation

Dissolve the components in the water. Sterilize by filtration.

5.3.3 Complete medium

5.3.3.1 Composition

Basic medium (5.3.1)	1 000 ml
Antibiotic solution (5.3.2)	5 ml

5.3.3.2 Preparation

Add the antibiotic solution to the basic medium, cooled down to $47\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$, then mix carefully. Pour about 15 ml of the complete medium into sterile Petri dishes (6.8). Allow to solidify. Immediately before use, carefully dry the agar plates, preferably with the lids off and the agar surface downwards, in a drying cabinet (6.2) for 30 min or until the agar surface is free of visible moisture. If they have been prepared in advance, the undried agar plates shall be kept for not more than 4 h at ambient temperature, or in the dark at $5\text{ }^{\circ}\text{C} \pm 3\text{ }^{\circ}\text{C}$ for not more than 7 days.

5.3.3.3 Performance testing

For the definition of selectivity and productivity, refer to ISO/TS 11133-1. For the performance criteria, refer to ISO/TS 11133-2:2003, Table B.5.

5.4 Columbia blood agar

5.4.1 Basic medium

5.4.1.1 Composition

Enzymatic digest of animal tissues	23,0 g
Starch	1,0 g
Sodium chloride	5,0 g
Agar	8,0 g to 18,0 g ^a
Water	1 000 ml

^a Depending on the gel strength of the agar.

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5.4.1.2 Preparation

Dissolve the basic components or the dehydrated complete medium in the water, by bringing to the boil. Adjust the pH, if necessary, so that after sterilization it is $7,3 \pm 0,2$ at $25\text{ }^{\circ}\text{C}$. Dispense the basic medium into flasks of suitable capacity. Sterilize in the autoclave (6.1) set at $121\text{ }^{\circ}\text{C}$ for 15 min.

5.4.2 Sterile defibrinated sheep blood

5.4.3 Complete medium

5.4.3.1 Composition

Basic medium (5.4.1)	1 000 ml
Sterile defibrinated sheep blood (5.4.2)	50 ml

5.4.3.2 Preparation

Add the blood aseptically to the basic medium, cooled down to $47\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$, then mix. Pour about 15 ml of the complete medium into sterile Petri dishes (6.8). Allow to solidify. Immediately before use, carefully dry the agar plates, preferably with the lids off and the agar surface downwards, in a drying cabinet (6.2) for 30 min or until the agar surface is free of visible moisture. If they have been prepared in advance, the undried agar plates shall be kept for not more than 4 h at ambient temperature, or not more than 7 days at $5\text{ }^{\circ}\text{C} \pm 3\text{ }^{\circ}\text{C}$.

5.4.3.3 Performance testing

For the definition of selectivity and productivity, refer to ISO/TS 11133-1.

For the performance testing, refer to ISO/TS 11133-2. Control strains *C. coli* ATCC 43478 or *C. jejuni* ATCC 33291 shall show good growth on Columbia blood agar after microaerobic incubation for 24 h at 37 °C.

5.5 Brucella broth

5.5.1 Composition

Enzymatic digest of casein	10,0 g
Enzymatic digest of animal tissues	10,0 g
Glucose	1,0 g
Yeast extract	2,0 g
Sodium chloride	5,0 g
Sodium hydrogen sulfite	0,1 g
Water	1 000 ml

5.5.2 Preparation

Dissolve the basic components or the dehydrated complete medium in the water, by heating if necessary. Adjust the pH, if necessary, so that after sterilization it is $7,0 \pm 0,2$ at 25 °C. Dispense the medium in quantities of 10 ml into tubes of suitable capacity. Sterilize in the autoclave (6.1) set at 121 °C for 15 min.

5.5.3 Performance testing

For the definition of selectivity and productivity, refer to ISO/TS 11133-1. For the performance criteria, refer to ISO/TS 11133-2:2003, Table B.4.

5.6 Reagent for the detection of oxidase

5.6.1 Composition

<i>N,N,N',N'</i> -Tetramethyl-1,4-phenylenediamine dihydrochloride	1,0 g
Water	100 ml

5.6.2 Preparation

Dissolve the component in the water immediately prior to use.

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.