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Kakovost vode - Ugotavljanje prisotnosti in števila toplotno obstojnih vrst Campylobacter

Water quality - Detection and enumeration of thermotolerant Campylobacter species

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Water quality — Detection and enumeration of thermotolerant *Campylobacter* species

Qualité de l'eau — Recherche et dénombrement d'espèces thermotolérantes du genre Campylobacter

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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 17995 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 4, *Microbiological methods*.

Introduction

Campylobacter jejuni subsp. *jejuni* and *Campylobacter coli* are common causes of intestinal infections in humans. *Campylobacter upsaliensis* may be of like importance. *Campylobacter lari* is less frequently associated with human infections. The vehicles for campylobacter infections are usually food, farm animals, pets and person-to-person contact, but water is also important.

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WARNING — Persons using this International Standard should be familiar with normal laboratory practice. This standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

IMPORTANT — It is absolutely essential that tests conducted in accordance with this International Standard be carried out by suitably trained staff.

1 Scope

This International Standard specifies a method for the detection and semiquantitative enumeration of thermotolerant *Campylobacter* species. The method can be applied to all kinds of filterable waters.

NOTE 1 The method can also be used as a presence/absence test for *Campylobacter* species in a specified sample volume.

NOTE 2 A more quantitative result can be obtained using a most probable number (MPN) set-up (see ISO 8199).

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 3696:1987, *Water for analytical laboratory use — Specification and test methods*

ISO 8199, *Water quality — General guidance on the enumeration of micro-organisms by culture*

ISO 19458, *Water quality — Sampling for microbiological analysis*

3 Terms and definitions

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

3.1
thermotolerant *Campylobacter* species
bacteria retained on filters during the filtration described in 8.2, multiplying during the selective enrichment described in 8.3, forming typical colonies during incubation at elevated temperatures on the selective medium described in 8.4, forming no visible colonies during incubation in air under the conditions specified in 8.6, being highly motile, slender rods with spiral morphology and a motility characterized by darting or corkscrew-like movements.

NOTE 1 Thermotolerant *Campylobacter* species of relevance in human infections include *Campylobacter jejuni* subsp. *jejuni* (hereafter referred to as *C. jejuni*), *C. coli*, *C. lari* and possibly *C. upsaliensis*.

NOTE 2 Thermotolerant *Campylobacter* species are Gram-negative, oxidase-positive and catalase-positive (strains of *C. upsaliensis* are reported to be catalase-negative or weakly positive) curved or spiral-shaped rods with a characteristic darting, often rotating, motility. In older cultures, coccoid forms occur.

NOTE 3 *Campylobacter* species require enriched media for optimum growth. They are very sensitive to toxic oxygen derivatives like peroxides and superoxide anions which can arise in media exposed to light and oxygen. They are microaerophilic and prefer an atmosphere containing approximately 5 % oxygen and approximately 10 % carbon dioxide (CO_2). Some *Campylobacter* species may require an atmosphere containing hydrogen.

NOTE 4 Most *C. jejuni*, *C. coli* and *C. lari* grow at temperatures between 32 °C and 45 °C, but some strains will not grow below 35 °C. Other strains may not grow above 43 °C.

NOTE 5 *C. upsaliensis* may not grow under the conditions described in this International Standard.

4 Principle

The sample is filtered through membrane filters with a pore size of 0,45 µm. The filters are transferred to selective enrichment broths and incubated for (44 ± 4) h at (37 ± 1) °C in a microaerobic environment. Following incubation, inoculums from each broth are streaked onto a solid medium, modified charcoal cefoperazone desoxycholate agar (mCCDA), and incubated for (44 ± 4) h at (41,5 ± 1) °C in a microaerobic environment. Colonies resembling campylobacters are tested for growth aerobically and, if negative, examined by microscopy. If necessary, further biochemical reactions are performed. See flow diagram in Annex A.

If typical campylobacters are found, the sample is positive for thermotolerant campylobacters. The result is given as the semiquantitative estimate per volume of sample (see Annex B).

Non-filterable waters can be analysed by direct inoculation of sample into enrichment broths. The ratio of sample to enrichment broth shall be 10 % or less.

NOTE When sufficient numbers of campylobacters are present, the sample can be streaked directly onto a solid medium, mCCDA, without prior selective enrichment.

5 Apparatus

5.1 Incubators, thermostatically controlled at (37 ± 1) °C and at (41,5 ± 1) °C.

5.2 Equipment for membrane filtration, as specified in ISO 8199.

5.3 Membrane filters: Sterile membrane filters made of cellulose ester with a diameter of 45 mm to 50 mm and a pore size of 0,45 µm.

Similar filters with a pore size of 0,22 µm are recommended for sterilization of supplements.

5.4 Equipment for microaerobic incubation: Jars able to maintain a modified atmosphere during incubation, fitted with valves for outlet and inlet of gases; vacuum pump; monitor for gas composition; and a suitable source of nitrogen, oxygen, carbon dioxide and preferably also hydrogen.

NOTE Commercially available equipment (like ANOXOMAT[1)]) can reproducibly deliver the modified atmosphere to the jars.

1) ANOXOMAT is a trade name. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

Alternatively, gas-generating pouches can be used if they are able to maintain an atmosphere with approximately 5 % oxygen, approximately 10 % carbon dioxide and preferably also approximately 10 % hydrogen.

5.5 Microscope, preferably with phase contrast.

5.6 Bottles, 150 ml to 250 ml, with airtight screw caps for the selective enrichments.

5.7 Vented Petri dishes, sterile, 9 cm.

5.8 Usual laboratory equipment.

6 Culture media and reagents

All ingredients and chemicals shall be of recognized quality, “for microbiology” or better. Water used shall be distilled or of like quality, as specified for ISO 3696:1987, grade 3. Follow the instructions given in Annex C.

Use of commercially available dehydrated substrates is encouraged, provided they comply with the descriptions in Annex C. They shall be prepared in accordance with the manufacturer's instructions.

Other grades of chemicals may be used provided they can be shown to lead to the same results.

7 Sampling, transport and storage

In addition to the instructions given in ISO 19458, be aware that campylobacters are very sensitive to adverse conditions. Keep samples cool (3 ± 2) °C and in the dark until the filtrations have been done. Avoid unnecessary mixing with air. Filter the samples as soon as possible after collection. Store for a maximum of 30 h prior to analysis.

NOTE Campylobacters survive well in clean water at (3 ± 2) °C. At higher temperatures or in other media, they may quickly deteriorate.

8 Procedure

8.1 General

Parallel to samples, run a positive control spiked in sample material or in sterile water through all the steps of the procedure to demonstrate the proper functioning of the apparatus, culture media and procedure, and to facilitate recognition of campylobacters (8.5 to 8.6).

Parts of each sample shall be enriched in the highly selective Preston broth (C.1.1) and parts in the less selective Bolton broth (C.1.2).

NOTE Preston broth may be too selective to allow the recovery of some strains of *C. coli*. Bolton broth may not be selective enough to counteract the growth of non-campylobacters in some samples. If the available sample size is limited, the most appropriate enrichment broth should be used. For waters with an expected high background count, it is more appropriate to use Preston broth, and for clean waters or where the background count is likely to be low Bolton broth is more appropriate.

NOTE 2 The amount of sample to be analysed varies with the sample material and the scope of the investigation. In Annex B, sample volumes for the analyses of drinking water are proposed.