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BSI Standards Publication

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

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National foreword

This British Standard is the UK implementation of ISO 17995:2019.

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

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

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Foreword

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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).

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For an explanation of the voluntary nature of standards, 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 www.iso.org/iso/foreword.html.

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

This second edition cancels and replaces the first edition (ISO 17995:2005), which has been technically revised. The main changes compared to the previous edition are as follows:

— the inclusion of direct inoculation of enrichment broths in addition to membrane filtration with additional information about sample processing.

— methods for the speciation of *Campylobacter*.

— performance testing for the quality assurance of culture media has been added to Annex D.

— performance characteristics of the method have been added as an Annex E.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

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Introduction

The thermotolerant *Campylobacter* species are not thought to propagate as free living but are zoonotic pathogenic bacteria of mammals and birds and which cause disease in humans. *Campylobacter jejuni* subsp. *jejuni* and *Campylobacter coli* are common causes of intestinal infections in humans. *Campylobacter upsaliensis* is found mainly in cats and dogs and is of minor importance for human infections. *Campylobacter lari* is less frequently associated with human infections. *Campylobacter* infections give rise to a flu-like illness with malaise, fever and myalgia followed by diarrhoea. The vehicles for *Campylobacter* infections are usually food, farm animals, pets and person-to-person contact; water is also important. They can be isolated from waters contaminated with human or animal faeces such as wastewater and surface waters. The bacteria have been demonstrated to survive within amoebae. Outbreaks of campylobacteriosis have been reported in relation to the use of contaminated drinking water and sporadic cases from recreational water use.

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

WARNING — Persons using this document should be familiar with normal laboratory practice. This document 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.

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

1 Scope

This document specifies a method for the detection, semi-quantitative and quantitative (MPN) enumeration of thermotolerant *Campylobacter* species.

The method can be applied to all kinds of waters including: drinking water, ground water and well water, fresh, brackish and saline surface water, swimming pools, spa and hydrotherapy pools, recreational waters, agricultural waters and runoff, untreated and treated wastewater and also sand and other sediments.

This method can be used for the detection of *Campylobacter* species in a specified sample volume. Clean water samples with low turbidity can be membrane filtered for either a qualitative method, semi-quantitative or quantitative (MPN) method. Water samples with higher turbidity, such as primary and secondary wastewater effluents and sediments, are analysed using the same qualitative, semi-quantitative or quantitative MPN method by direct inoculation of material into bottles or tubes. Sediments can be suspended in a suitable diluent or inoculated directly into enrichment broths.

Users wishing to employ this method are expected to verify its performance for the particular matrix under their own laboratory conditions.

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 8199, *Water quality — General requirements and guidance for microbiological examinations by culture*

ISO 11133, *Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media*

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

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:

— ISO Online browsing platform: available at https://www.iso.org./obp

— IEC Electropedia: available at http://www.electropedia.org/

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3.1
Campylobacter
microorganisms forming characteristic colonies on solid selective media when incubated in a microaerobic atmosphere at 41,5 °C and which possess the characteristic morphology, motility, biochemical and growth properties described when tests are conducted in accordance with ISO 17995

Note 1 to entry: Thermotolerant *Campylobacter* species of relevance in human infections include *Campylobacter jejuni* subsp. *jejuni* (hereafter referred to as *Campylobacter jejuni*), *Campylobacter coli*, *Campylobacter lari* and possibly *Campylobacter upsaliensis*. *Campylobacter upsaliensis* is found mainly in cats and dogs and is of minor importance for human infection.

3.2
detection of *Campylobacter*
determination of the presence or absence, semi-quantitative enumeration or quantitative enumeration using a most probable number (MPN) system of *Campylobacter* in water and solid materials when the test is conducted in accordance with ISO 17995

4 Principle

4.1 General

In general, the detection of *Campylobacter* requires enrichment followed by isolation of colonies and their confirmation. The flow diagram of the procedure in Annex A shall be applied.

4.2 Inoculation and enrichment in selective liquid medium

Samples are inoculated either directly or after concentration using membrane filtration into one of two selective enrichment broths depending on the expected level of background microorganisms: Bolton broth for clean water and Preston broth for more heavily contaminated water. A single sample volume is processed for *Campylobacter* detection and, where necessary, at least three 10-fold volumes (for example 10 ml, 100 ml and 1 000 ml) are used for a semi-quantitative determination (Annex B shall be applied). For a quantitative (MPN) determination, volumes of 500 ml, 5 × 100 ml, 5 × 10 ml and, where counts may be high, smaller volumes are used or the initial sample is diluted. The broths are then incubated microaerobically at (37 ± 1) °C for (44 ± 4) h.

The ratio of sample to enrichment broth shall be 10 % or less if single strength broth is used.

4.3 Isolation and selection for confirmation

From the enrichment broth cultures in 4.2, liquid selective media are inoculated onto modified charcoal cefoperazone deoxycholate agar (mCCDA).

The mCCDA plates are then incubated at (41,5 ± 1) °C for (44 ± 4) h in a microaerobic atmosphere and examined for characteristic colonies after incubation. Colonies with typical characteristics are presumed to be *Campylobacter* (see 9.3). It is necessary to confirm at least one colony from each plate to demonstrate that the corresponding enrichment culture is positive for the presence of *Campylobacter* species.

4.4 Confirmation

Confirmation of suspect colonies of *Campylobacter* species involves biochemical, morphological and physiological tests. See flow diagram in Annex A.

The colonies presumed to be *Campylobacter* are inoculated on the non-selective Columbia agar or other appropriate solid non-selective media, and are then confirmed by means of microscopic examination, failure to grow aerobically at 25 °C and appropriate biochemical tests. Optionally, *Campylobacter* species are further identified by specific biochemical tests.

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For species identification, it is recommended that more than one isolate per enrichment culture is tested.

If typical *Campylobacter* species are confirmed or identified, the result is given as the qualitative, semi-quantitative or quantitative (MPN) estimate per volume of sample.

5 Culture media and reagents

For current laboratory practice see ISO 8199 and ISO 11133.

The composition and preparation of the culture media and reagents given in Annex C shall be used. The performance testing requirements given in Annex D shall be applied.

6 Apparatus

Usual microbiological laboratory equipment (as specified in ISO 8199) and, in particular, the following:

6.1 Water baths or incubators, capable of operating at (37 ± 1) °C and (41,5 ± 1) °C.

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

6.3 Membrane filtration equipment, as specified in ISO 8199.

6.4 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. Larger diameter membranes may be used with suitable filter holders provided they have a pore size of 0,45 µm.

6.5 pH-meter, accurate to within 0,2 pH units at 25 °C.

6.6 Apparatus suitable for achieving a microaerobic atmosphere, with oxygen content of (5 ± 2) %, carbon dioxide (10 ± 3) %, optional hydrogen at ≤10 %, with the balance being nitrogen.

Appropriate gastight containers of, for example, 3,5 l capacity are used to hold Petri dishes and/or flasks or bottles for the enrichment broth, such as bacteriological anaerobic jars. 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. The jar may be flushed and filled with an appropriate gas mixture prior to incubation. Alternatively, an incubator with appropriately modified atmosphere (approximately 5 % oxygen and 10 % carbon dioxide) can also be used.

Gas-generating pouches can be used if they are able to maintain an atmosphere with approximately 5 % oxygen and approximately 10 % carbon dioxide.

6.7 Microscope, preferably with phase contrast or differential interference contrast. Dark ground illumination can also be used.

6.8 Bottles, 100 ml to 250 ml, with screw caps for the selective enrichments.

6.9 Vented Petri dishes, sterile, 90 mm.

6.10 Disinfected forceps, for handling membrane filters.

6.11 Refrigerator, capable of operating at (5 ± 3) °C.

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