

Australian/New Zealand Standard™

AS/NZS 4276.19

Water microbiology

Method 19: Examination for thermophilic *Campylobacter* spp.—Membrane filtration

PREFACE

This Standard was prepared by the Joint Standards Australia/Standards New Zealand Committee FT-020, Water Microbiology, as part of a series of methods for the microbiological examination of waters for domestic and industrial use.

The genus *Campylobacter* comprises two main groups according to growth temperature. The first group will grow at 42°C and are referred to as thermophilic *Campylobacter*s. The second group will grow at 25°C but not at 42°C. Thermophilic *Campylobacter*s include the important human pathogens *C.jejuni* and *C.coli* and details of their method of isolation from waters are covered by this Standard.

The development and availability of PCR systems for the detection and identification of *Campylobacter* may lead to the use of a standardized PCR technique as an adjunct to this method. These tests would not be within the capability of an otherwise well-equipped microbiological laboratory and are not included in this Standard. Laboratories that cannot perform these specialized tests should consult a laboratory with the necessary facilities if further testing, beyond the scope of this Standard, is required to establish the identity of an organism isolated by this method.

The term ‘informative’ has been used in this Standard to define the application of the appendix to which it applies. An ‘informative’ appendix is only for information and guidance.

METHOD

1 SCOPE

This Standard sets out a method for detecting thermophilic *Campylobacter*s in water using culture in enrichment broth and on selective agar media.

NOTE: A flow diagram of the procedure is shown in Appendix A.

2 REFERENCED DOCUMENT

The following document is referred to in this Standard:

AS	
4276	Water microbiology
4276.1	Method 1: General information and procedures

3 PRINCIPLE

Bacteria in the genus *Campylobacter* are micro-aerobic, requiring an oxygen tension of around 5% and a carbon dioxide tension of around 10%. Isolation of thermophilic *Campylobacters* is achieved by concentration and isolation of organisms on a membrane followed by broth enrichment and subculture to a selective agar. The broth and agar are both incubated micro-aerobically at 42°C. Confirmation of thermophilic *Campylobacters* is by cell morphology, Gram stain and oxidase test.

4 CULTURE MEDIA, REAGENTS AND REFERENCE CULTURES

4.1 Culture media (See Appendix B)

4.1.1 Preston enrichment broth

4.1.2 Prestons selective agar or a blood free modification of this medium

4.1.3 Blood agar

4.2 Reagent

Kovacs' oxidase reagent

4.3 Reference cultures

4.3.1 *Campylobacter jejuni* NCTC 11351 or ACM 3393 or NZRM 2397. This organism gives positive reactions for thermophilic *Campylobacter* spp.

4.3.2 *Escherichia coli* NCTC 9001 or ATCC 11775 or ACM 1803 or NZRM 3309. This organism gives negative reactions for thermophilic *Campylobacter* spp.

NOTES:

- 1 NCTC—National Collection of Type Cultures
ACM—Australian Collection of Microorganisms
NZRM—New Zealand Reference Culture Collection (Medical Section)
ATCC—American Type Culture Collection
- 2 The cultures referenced in Clauses 4.3.1 and 4.3.2 are obtainable from the following laboratories:

In Australia:

Australian Collection of Microorganisms (ACM)
Department of Microbiology
The University of Queensland
BRISBANE QLD 4072

In New Zealand:

New Zealand Reference Culture Collection (Medical Section) (NZRM)
ESR Kenepuru Science Centre
Kenepuru Drive
Porirua

4.3.3 Use of reference cultures

When testing a sample of water by this standard method, cultures of positive reference cultures shall be submitted to the test procedures at the same time to demonstrate and ensure that typical growth characteristics are exhibited by the reference culture.

5 APPARATUS

5.1 Gas cylinders or kits

To produce a micro-aerobic incubation atmosphere.

5.2 Filtration equipment and 0.2 µm or 0.45 µm membranes

47 mm diameter filter.

5.3 Incubators

Operating at $42 \pm 1^\circ\text{C}$.

6 PROCEDURES

6.1 Micro-aerobic incubation conditions

Campylobacters will only grow in a micro-aerobic environment. The micro-aerobic atmosphere comprises a mixture of 5% oxygen, 10% carbon dioxide and 85% nitrogen. Kits and gas cylinders (5.1) providing this atmosphere are available commercially. Since hydrogen is produced, jars should not be opened adjacent to a source of ignition.

6.2 Sample volume

The sample volume may vary according to the source and level of sensitivity desired, however a 1 L sample is recommended for most investigations.

6.3 Membrane filtration

Filter one to several litres of water through a membrane of pore size of 0.2 µm (5.2). However, for turbid water, 0.45 µm of pore size may be required. This may result in reduced recovery. In addition, in some circumstances pre-filtration may be necessary.

6.4 Enrichment

Place the membrane(s) in a suitable screw capped container filled with 50 mL of Preston enrichment broth (without antibiotic supplement). Larger volume is required for multiple membranes. Incubate under aerobic conditions at $36 \pm 1^\circ\text{C}$ for 2 h.

NOTES:

- 1 25 mL is found to be suitable for single membranes in most situations.
- 2 The surface area to volume ratio of the enrichment broth in the container should ensure that the airspace in the container is minimized.

6.5 Controls for broth culture

Inoculate a separate container of Preston broth (without antibiotic supplement) with the reference culture specified in Clause 4.3.

6.6 Selective enrichment

After the two hours incubation, add 0.4 mL of antibiotic supplement to each 100 mL of broth culture. Incubate the selective broths under micro-aerobic conditions (see Clause 6.1) at $42 \pm 1^\circ\text{C}$ for 48 ± 2 h.

6.7 Plating out on selective agar media

The procedure shall be as follows:

- (a) Subculture from the enrichment broth to Prestons selective agar or a blood free modification of this medium (See Clause 4.1.2).
- (b) Incubate the plates at $42 \pm 1^\circ\text{C}$ in a micro-aerobic atmosphere for 48 ± 2 h.

6.8 Examination of selective agar plates

Examine plates for characteristic colonies, using a hand lens if necessary. If suspect colonies have formed, perform confirmation tests specified in Clause 7.

NOTES:

- 1 *Campylobacter* colonies vary in size but are typically flat and watery with an entire edge and may be circular or ovoid. They have a tendency to spread along lines of inoculation.
- 2 Variation in colonial morphology is not uncommon so it is advisable to perform confirmation tests on several colonies.

7 CONFIRMATION TESTS

7.1 Culture of selected colonies

The procedure shall be as follows:

- (a) Subculture each selected colony onto a separate blood agar plate.
- (b) Incubate the inoculated plates in a micro-aerobic atmosphere (see Clause 6.1) at $42 \pm 1^\circ\text{C}$ for $24 \pm 2\text{h}$ or until growth is visible.

NOTE: A supplementary HBA plate incubated aerobically may be useful for screening purposes. *Campylobacter* species will not grow on HBA when incubated aerobically.

7.2 Oxidase test and Gram stain

The procedure shall be as follows:

- (a) Carry out an oxidase test on suspect colonies.
NOTE: *Campylobacters* are oxidase positive.
- (b) Carry out a Gram stain on oxidase-positive colonies using dilute carbol fuchsin as the counterstain because *Campylobacters* may stain weakly with other counter stains.

NOTE: *Campylobacters* produce Gram-negative curved rods which resemble gull's wings, S shapes or short spirals. They are in the size range $0.2\text{ }\mu\text{m}$ to $0.8\text{ }\mu\text{m}$ by $1.5\text{ }\mu\text{m}$ to $5\text{ }\mu\text{m}$.

7.3 Further confirmation tests

For most practical purposes colonial and cellular morphology and the oxidase test are adequate for identification. If required, species and subspecies differentiation can be carried out using the bio-typing scheme of Skirrow and Benjamin[7].

Additional tests for *Campylobacter* confirmation and identification may be employed and include commercial kits employing latex particles coated with polyvalent immunoglobulins and other techniques such as lectin agglutination, cellular fatty acids, profiles, nucleic acid probes, polymerase chain reactions and other genomic methods.

8 TEST REPORT

The report shall contain the following information:

- (a) Reference to this Australian/New Zealand Standard, i.e. AS/NZS 4276.19.
- (b) All details necessary for complete identification of the sample.
- (c) Dates of sample collection, receipt and test commencement.
- (d) The presence or absence of thermophilic *Campylobacters* in the sample volume tested.
- (e) Any circumstances or conditions which may have influenced the result.