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 Campylobacters. The second group will grow at 25°C but not at 42°C. Thermophilic Campylobacters 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 Campylobacters 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

Standards Australia
STANDARDS NEW ZEALAND Paerewa Aotearoa

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 ±1°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 ±1°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 ±1°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 ±1°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.