Australian Standard®

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

PREFACE

This Standard was prepared by the Australian members of Joint Standards Australia/Standards New Zealand Committee FT-020, Water Microbiology, to supersede AS/NZS 4276.19:2001, *Water microbiology*, Method 19: *Examination for thermophilic Campylobacter spp.—Membrane filtration*.

After consultation with stakeholders in both countries, Standards Australia and Standards New Zealand decided to develop this Standard as an Australian Standard rather than an Australian/New Zealand Standard.

The objective of this revision is to update the Standard and consider ISO 17995:2005, *Water quality—Detection and enumeration of thermotolerant Campylobacter species*, and research over the last 10 years into methods and media for the detection of *Campylobacter*.

ISO 17995:2005 has not been adopted due to this method's age and that work has commenced to technically review the first edition. In addition, laboratories in Australia require some level of speciation and ISO 17995:2005 does not include speciation nor a resuscitation step at enrichment prior to antibiotic addition. A review of recent publications suggests that mExeter broth is the enrichment medium of choice for waters with expected high background counts as it is more inhibitory compared to Bolton broth.

The genus *Campylobacter* comprises two main groups according to growth temperature. Those in the first group will grow at 42°C and are referred to as thermophilic *Campylobacter* spp. The second group will grow at 25°C but not at 42°C.

The thermophilic *Campylobacter* spp. are not thought to be free living but are obligate parasites of humans, animals and birds. *Campylobacter jejuni*, (*C. jejuni*) and *Campylobacter coli* (*C. coli*) are common causes of intestinal infections in humans. *Campylobacter upsaliensis* (*C. upsaliensis*) may be of like importance. *Campylobacter lari* (*C. 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 also be isolated from waters contaminated with human or animal faeces such as wastewater and surface waters. Outbreaks of campylobacteriosis have been reported in relation to the use of contaminated drinking water and from recreational water use.

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.

STANDARDS Australia

METHOD

1 SCOPE

This Standard sets out a method for detection and speciation of thermophilic Campylobacters in water using culture in enrichment broth and on selective agar media.

NOTE: Due to the selective nature of the isolation media used and its requirements for a hydrogen enhanced microaerobic atmosphere, some strains of *C. upsaliensis* may not be detected.

2 REFERENCED DOCUMENTS

The following documents are referred to in this Standard:

AS
2031 Water quality—Sampling for microbiological analysis (ISO 19458:2006, MOD)

4276 Water microbiology
4276.1 Method 1: General information and procedures

3 PRINCIPLE

Campylobacter spp. are microaerophilic microorganisms requiring reduced oxygen tension of approximately 5% and an increased carbon dioxide tension of approximately 10%. Isolation of thermophilic *Campylobacter* spp. is achieved by concentration of organisms on a membrane filter followed by broth enrichment and subculture onto a selective agar. The broth and agar are both incubated microaerobically at 41.5°C. Confirmation of *Campylobacter* spp. involves biochemical, morphological and physiological tests. Alternative confirmation may be achieved using genotypic tests.

NOTE: This method does not include enumeration. If enumeration of thermophilic *Campylobacter* spp. in water is required, a method that may be employed is the most probable number (MPN) method using the multiple tube dilution technique. See AS 4276.1. The volumes tested depend on the degree of contamination of the water, but the set of 50 mL, 10 mL and 1 mL is a useful starting point. The method commences with enrichment using Bolton or mExeter broth, and then proceeds according to this standard. Calculation of the most probable number per given volume requires the use of MPN tables.

4 CULTURE MEDIA AND REAGENTS

4.1 Culture media

4.1.1 *Modified Exeter broth (mExeter)*

4.1.2 *Bolton broth*

4.1.3 *Modified charcoal cefoperazone dexoxycholate agar (mCCD)*

4.1.4 *Blood agar*

4.2 Reagents

4.2.1 *Kovacs'oxidase reagent*

4.2.2 *Dilute carbol fuschin*

4.2.3 *Physiological saline solution, 0.85% aqueous solution*

4.2.4 *Sodium hippurate, 10 g/L aqueous solution*

4.2.5 *Ninhydrin solution, 3.5% in 1:1 mixture of acetone and butanol*

4.2.6 *Catalase reagent, 3% hydrogen peroxide (H_2O_2)*

4.2.7 *Indoxyl acetate discs*

