

Australian/New Zealand Standard™

Water microbiology

Method 7: *Escherichia coli* and thermotolerant coliforms—Membrane filtration method

PREFACE

This Standard was prepared by the Joint Standards Australia/Standards New Zealand Committee FT-020, Water Microbiology, to supersede AS 4276.7—1995, *Water microbiology, Method 7: Thermotolerant coliforms and Escherichia coli—Membrane filtration method*.

The objective of this revision is to extend the applicability of the membrane filtration method when used with water samples containing different levels of competing non-target bacteria flora.

The merits of ISO 9308-1:2000, *Water quality—Detection and enumeration of Escherichia coli bacteria, Part 1: Membrane filtration method* are acknowledged for waters containing very low numbers of background organisms. Should waters for analysis universally have a high likelihood of containing only few background organisms (e.g. high quality food processing waters, highly treated waters, etc), the ISO 9308-1:2000 method is a suitable choice. ISO 9308-1:2000 has been adopted as AS/NZS 4276.22: *Water microbiology, Method 22: Packaged water—Coliform bacteria and Escherichia coli—Membrane filtration method* and has been published concurrently with this Standard.

The Committee has restricted application of ISO 9308-1:2000 to packaged waters because this method is only applicable to waters with low background microflora and many waters in Australia and New Zealand contain background organisms at concentrations too high for the ISO method to be widely applicable.

Therefore, this Standard applies to testing for coliforms in all waters other than packaged waters. For packaged water use AS/NZS 4276.22. Where waters are expected to contain low to moderate numbers of bacteria, use of membrane lauryl sulfate medium (m-LS medium) is recommended as the primary isolation medium. Where waters have a likelihood of containing high numbers of background microorganisms (e.g. some ambient untreated waters, effluent waters), this Standard includes the option to employ an alternative selective medium (m-FC medium) that is generally more inhibitory and can be expected to a greater extent to restrict the number and size of background colonies.

Thermotolerant coliforms are gram-negative, non-spore-forming, rod shaped bacteria capable of aerobic and facultative anaerobic growth. They are a subset of coliform bacteria that are able to metabolise lactose at 44°C and are cytochrome oxidase negative. *Escherichia coli* (*E. coli*) are thermotolerant coliforms that, in addition to metabolising lactose at 44°C and being cytochrome oxidase negative, express the enzyme β -glucuronidase and produce indole from tryptophan.

The laboratory should have a clearly defined quality control system to ensure that the apparatus, culture media, reagents and technique are suitable for the test. The use of positive controls is part of this system.

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, using membrane filtration, for enumerating *Escherichia coli* (*E. coli*) and thermotolerant coliforms in water other than packaged water.

Where waters are expected to contain low to moderate numbers of background bacteria, use of membrane lauryl sulfate agar or broth (m-LS) is recommended as the primary isolation medium. Where waters have a likelihood of containing high numbers of background microorganisms (e.g. some ambient untreated waters, effluent waters), this Standard includes the option to employ an alternative selective agar or broth (m-FC) that is generally more inhibitory and can be expected to a greater extent to restrict the number and size of background colonies.

NOTES:

- 1 Thermotolerant coliforms are often also referred to as ‘faecal coliforms’ although the organisms may not be of faecal origin.
- 2 Membrane filtration is suitable for enumerating microorganisms only when the turbidity of the water is low.
- 3 A flow diagram of the procedure is shown in Appendix A.

2 REFERENCE DOCUMENTS

The following document is referred to in this Standard:

AS/NZS
4276 Water microbiology
4276.1 Method 1: General information and procedures (ISO 8199:2005, MOD)

3 DEFINITIONS

For the purpose of this Standard the following definitions apply:

3.1 Thermotolerant coliforms

Bacteria which are capable of producing gas from lactose-based media at $44.0 \pm 0.5^\circ\text{C}$ within 21 ± 3 hours.

3.2 *E. coli*

A thermotolerant coliform that expresses the enzyme β -glucuronidase, hydrolysing the substrate 4-methylumbelliferyl- β -D-glucuronide (MUG) to produce a blue fluorescence when viewed under long wavelength ultra-violet light.



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