



## Water microbiology

### Method 5: Coliforms, *Escherichia coli* and thermotolerant coliforms— Membrane filtration method

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Australia



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## Preface

This Standard was prepared by the Standards Australia Committee FT-020, Water Microbiology, to supersede AS/NZS 4276.5:2007, *Water microbiology, Method 5: Coliforms—Membrane filtration method* and AS/NZS 4276.7:2007, *Water microbiology, Method 7: Escherichia coli and thermotolerant coliforms—Membrane filtration method*.

The objective of this Standard is to specify a method, using membrane filtration, for enumerating coliforms, *Escherichia coli* (*E. coli*) and thermotolerant coliforms in water other than packaged water.

The major changes in this edition are as follows:

- (a) Combine the methods of AS/NZS 4276.5 with AS/NZS 4276.7.
- (b) Incorporate chromogenic selective media that are capable of enumerating coliforms, *Escherichia coli* and thermotolerant coliforms in a more timely manner.
- (c) Incorporate culture media and reagents.
- (d) Remove reference to AS 4276.2.
- (e) Update reference cultures.
- (f) Update the normative and informative references.

The terms “normative” and “informative” are used in Standards to define the application of the appendices to which they apply. A “normative” appendix is an integral part of a Standard, whereas an “informative” appendix is only for information and guidance.

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## Introduction

The merits of the United States Environmental Protection Agency (USEPA) Method 1604: *Total Coliforms and Escherichia coli in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium)*, 2002, have been considered by the committee and this method has been adopted in part for this procedure. The USEPA method is primarily used for the microbial analysis of potable water. The method states that it is also suitable for recreational, surface, marine water, bottled water, ground water, sewage effluent, and environmental waters. The method has only been validated for the enumeration of total coliforms and *E. coli* in drinking water by membrane filtration.

Coliforms are Gram negative, non-spore-forming, rod-shaped bacteria capable of aerobic and facultative anaerobic growth. They metabolize lactose at 36 °C, expressing the enzyme  $\beta$ -galactosidase and are cytochrome oxidase negative. Coliforms are comprised of several species from genera within the family Enterobacteriaceae, including *E. coli*. The coliform group includes species of both faecal and environmental origin.

Thermotolerant coliforms are a sub-group of coliforms that are able to grow at 44 °C  $\pm$  0.5 °C. Thermotolerant coliforms are also referred to as “faecal coliforms” although the organisms may not be of faecal origin.

Two enzyme substrates are included in the medium used in this method to detect the enzymes  $\beta$ -galactosidase and  $\beta$ -glucuronidase, produced by most strains of coliforms and *E. coli* bacteria, respectively.

# Australian Standard<sup>®</sup>

## Water microbiology

### Method 5: Coliforms, *Escherichia coli* and thermotolerant coliforms—Membrane filtration method

#### 1 Scope

This Standard sets out a method, using membrane filtration, for enumerating coliforms, *E. coli* and thermotolerant coliforms in water other than packaged water.

For the enumeration of total coliforms and *E. coli* where waters are expected to contain low numbers of bacteria (e.g. potable waters, treated water), this method provides the option to use either MI agar or chromogenic *E. coli*/coliform selective agar, with incubation at  $36\text{ °C} \pm 2.0\text{ °C}$ .

For the enumeration of thermotolerant coliforms or *E. coli* where waters are expected to contain high numbers of bacteria (e.g. untreated waters, surface waters and effluents), the use of Chromogenic *E. coli*/coliform selective agar incubated at  $44\text{ °C} \pm 0.5\text{ °C}$  is required. MI agar is not suitable for samples with high bacterial load due to the likelihood of diffusion of fluorescence of colonies on membranes with high counts.

Both media used in this method have the ability to simultaneously detect and enumerate total coliforms, *E. coli* and thermotolerant coliforms in water samples in 24 h or less, on the basis of their specific enzyme activities.

MI agar contains two enzyme substrates:

- (a) fluorogenic 4-Methylumbelliferyl- $\beta$ -D-galactopyranoside (MUGal); and
- (b) a chromogen Indoxyl- $\beta$ -D-glucuronide (IBDG),

to detect the enzymes  $\beta$ -galactosidase and  $\beta$ -glucuronidase, produced by most coliforms and *E. coli*, respectively.

Chromogenic *E. coli*/coliform selective agar contains two chromogenic agents:

- (i) 6-chloro-3-indolyl- $\beta$ -D-galactopyranoside (Rose-Gal) that detects  $\beta$ -galactosidase activity of the coliform group; and
- (ii) 5-bromo-4-chloro-3-indolyl- $\beta$ -D-glucuronide cyclohexylammonium salt (X-Glu) that detects  $\beta$ -glucuronidase activity in *E. coli* bacteria.

NOTE 1 Membrane filtration is suitable for enumerating microorganisms only when the turbidity of the water is low.

NOTE 2 Historically, thermotolerant coliforms, previously referred to as faecal coliforms, were considered to be faecally derived. More recent evidence indicates *E. coli* is a better indicator of faecal contamination.

NOTE 3 A flow diagram of the procedure is shown in [Appendix A](#).

#### 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:

AS 5140, *Microbiology of food, animal feed and water—Preparation, production, storage and performance testing of culture media (ISO 11133:2014, MOD)*

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