AS 4276.5:2019

Australian STANDARD

Water microbiology

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

STANDARDS Australia

AS 4276.5:2019

This Australian Standard® was prepared by FT-020, Water Microbiology. It was approved on behalf of the Council of Standards Australia on 12 February 2019.

This Standard was published on 4 March 2019.

The following are represented on Committee FT-020:

ACT Health
Australian Society for Microbiology
Australian Water Association
Chemistry Australia
National Association of Testing Authorities Australia
National Measurement Institute
NSW Health
PathWest Laboratory Medicine, WA
Queensland Health
Queensland University of Technology
University of Melbourne
Victorian Water Industry Association
Water NSW
Water Services Association of Australia

This Standard was issued in draft form for comment as DR AS 4276.5:2018.

Keeping Standards up-to-date

Ensure you have the latest versions of our publications and keep up-to-date about Amendments, Rulings, Withdrawals, and new projects by visiting:
www.standards.org.au

ISBN 978 1 76072 392 7

Australian STANDARD

Water microbiology

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

Originated as AS 1095.4.1.5—1981.
Previous edition AS 4276.5—1995.
Jointly revised and designated as AS/NZS 4276.5:2007.
Amalgamated with AS/NZS 4276.7:2007 and revised and redesignated as AS 4276.5:2019.

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.

Contents

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 β-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 ± 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 β-galactosidase and β-glucuronidase, produced by most strains of coliforms and *E. coli* bacteria, respectively.

Australian Standard®

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 °C ± 2.0 °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 °C ± 0.5 °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-β-D-galactopyranoside (MUGal); and

(b) a chromogen Indoxyl-β-D-glucuronide (IBDG),

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

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

(i) 6-chloro-3-indolyl-β-D-galactopyranoside (Rose-Gal) that detects β-galactosidase activity of the coliform group; and

(ii) 5-bromo-4-chloro-3-indolyl-β-D-glucuronide cyclohexylammonium salt (X-Glu) that detects β-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)*