STANDARDS AUSTRALIA/STANDARDS NEW ZEALAND

RECONFIRMATION

OF
AS/NZS 4276.6:2007
Water microbiology
Method 6: Coliforms, *Escherichia coli* and thermotolerant coliforms–
Determination of most probable number (MPN)

RECONFIRMATION NOTICE

Technical Committee FT-020 has reviewed the content of this publication and in accordance with Standards Australia procedures for reconfirmation, it has been determined that the publication is still valid and does not require change.

Certain documents referenced in the publication may have been amended since the original date of publication. Users are advised to ensure that they are using the latest versions of such documents as appropriate, unless advised otherwise in this Reconfirmation Notice.

Approved for reconfirmation in accordance with Standards Australia procedures for reconfirmation on 16 August 2017.

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The following are represented on Technical Committee FT-020:

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NOTES

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Australian/New Zealand Standard™

Water microbiology

Method 6: Coliforms, *Escherichia coli* and thermotolerant coliforms—Determination of most probable number (MPN)

PREFACE

This Standard was prepared by the Joint Standards Australia/Standards New Zealand Committee FT-020, Water Microbiology, to supersede AS 4276.4—1995, *Water microbiology,* Method 4: *Coliforms—Estimation of most probable number (MPN)* and AS 4276.6—1995, *Water microbiology*, Method 6: *Thermotolerant coliforms and Escherichia coli—Estimation of most probable number (MPN).*

The objective of this revision is to combine the most probable number method for coliforms with that of thermotolerant coliforms and *Escherichia coli* to form a single method.

The Committee has not recommended the adoption of ISO 9308-2:1990, *Water quality—Detection and enumeration of coliform organisms, thermotolerant coliform organisms and presumptive Escherichia coli*—Part 2*: Multiple tube (most) probable number method*, because at the time of review the ISO method was considered to be outdated. In addition, there was a desire to align the revision of AS 4276.4—1995 and AS 4276.6—1995 employing most probable number technique with that of the membrane filtration methods, AS 4276.5 and AS 4276.7, in moving toward an enzyme-based definition and confirmation for coliforms and *Escherichia coli*.

Coliforms are gram-negative, non-spore-forming, rod shaped bacteria capable of aerobic and facultative anaerobic growth. They metabolize lactose at 36°C, express the enzyme β-galactosidase and are cytochrome oxidase negative. Coliforms comprise several species from genera within the family Enterobacteriaceae, including *Escherichia coli*, and some species of *Enterobacter*, *Klebsiella*, *Citrobacter*, *Serratia* and *Hafnia*. The Coliform group includes species of both faecal and environmental origin.

Thermotolerant coliforms are a subset of coliform bacteria that are able to metabolise lactose at 44°C. *Escherichia coli* (*E. coli*) are thermotolerant coliforms that, in addition to metabolizing lactose at 44°C, express the enzyme β-glucuronidase and produce indole from trytophan.

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.

STANDARDS Australia
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METHOD

1 SCOPE

This Standard sets out a method, using a multiple tube dilution technique, for determining the most probable number (MPN) of coliforms, *Escherichia coli (E. coli)* and thermotolerant coliforms in water. It is suitable for waters with high background flora and/or turbidity.

The method is suitable for non-food solid matrices. The preparation of such matrices are beyond the scope of this method (see Clause 12, Bibliography for further guidance).

NOTES:

1 Thermotolerant coliforms are also referred to as 'faecal coliforms' although the organisms may not be of faecal origin.

2 A flow diagram of the procedure is shown in Appendix A.

2 REFERENCED DOCUMENTS

The following documents are referred to in this Standard:

AS/NZS
4276 Water microbiology
4276.1 Method 1: General information and procedures

3 DEFINITIONS

For the purpose of this Standard the following definitions apply:

3.1 Coliforms

Organisms capable of growth at 36 ±2°C, which express the enzyme β-galactosidase and are oxidase negative.

3.2 Thermotolerant coliforms

Organisms which are capable of producing gas from lactose-based media at 44.0 ±0.5°C within 21 ±3 hours.

3.3 *Escherichia coli (E. coli)*

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

4 PRINCIPLE

Measured quantities of sample, of one or more dilutions, are distributed into a series of tubes (or bottles) of liquid medium containing lactose and bromocresol purple as an indicator of acidity. Upon incubation, each tube which received one or more of the target microorganisms in the inoculum will display characteristic growth. Presumptive positive tubes are sub-cultured onto confirmatory media.

Coliform express the enzyme β-galactosidase and are negative for cytochrome oxidase. *E. coli* is confirmed by the expression of the enzyme β-glucuronidase in EC-MUG broth incubated at 44°C. Thermotolerant coliforms are confirmed on the basis of turbidity and gas production in EC-MUG incubated at 44°C.

Providing there are some tubes that exhibit a negative reaction, the most probable number of target microorganisms in 100 mL of the sample is determined from the number and distribution of tubes displaying confirmed positive reactions.