Australian Standard®

Water microbiology

Method 21: Examination for coliforms and *Escherichia coli*—Determination of most probable number (MPN) using enzyme hydrolysable substrates (ISO 9308-2:2012, MOD)

PREFACE

This Standard was prepared by the Standards Australia Committee FT-020, Water Microbiology, to supersede AS 4276.21—2005, *Water microbiology*, Method 21: *Examination for coliforms and Escherichia coli—Determination of most probable number (MPN) using enzyme hydrolysable substrates*.

The objective of this Standard is to specify a method for the enumeration of *Escherichia coli* (*E. coli*) and coliform bacteria in water.

This method is not to be used for the enumeration of *E. coli* and coliform bacteria in marine water.

This Standard is an adoption with national modifications and has been reproduced from ISO 9308-2:2012, *Water quality — Enumeration of Escherichia coli and coliform bacteria*, Part 2: *Most probable number method*.

Appendix ZZ lists the variations to ISO 9308-2:2012 for the application of this Standard in Australia.

Appendix ZA provides additional information for Australian conditions.

As this Standard is reproduced from an International Standard, the following applies:

(a) In the source text 'this part of ISO 9308' should read 'this Australian Standard'.

(b) A full point substitutes for a comma when referring to a decimal marker.

(c) Substitute 'ml' for 'ml' wherever it appears.

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

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INTRODUCTION

The presence and extent of faecal pollution is an important factor in assessing the quality of a body of water and the risk to human health from infection. Examination of water samples for the presence of *Escherichia coli (E. coli)*, which normally inhabits the bowel of man and other warm-blooded animals, provides an indication of such pollution. Examination for coliform bacteria can be more difficult to interpret because some coliform bacteria live in soil and surface fresh water and are not always intestinal. Therefore, the presence of coliform bacteria, although not a proof of faecal contamination, may indicate a failure in treatment or ingress of water into the distribution system.

The International Organization for Standardization (ISO) draws attention to the fact that it is claimed that compliance with this document may involve the use of patents concerning Colilert-18 and Quanti-Tray and Quanti-Tray 2000 given in this document.

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AUSTRALIAN STANDARD

Water microbiology

Method 21: Examination of coliforms and *Escherichia coli*— Determination of most probable number (MPN) using enzyme hydrolysable substrates (ISO 9308-2:2012, MOD)

WARNING – Persons using this part of ISO 9308 should be familiar with normal laboratory practice. This International Standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

IMPORTANT – It is absolutely essential that tests conducted in accordance with this part of ISO 9308 be carried out by suitably qualified staff.

1 Scope

This part of ISO 9308 specifies a method for the enumeration of *E. coli* and coliform bacteria in water. The method is based on the growth of target organisms in a liquid medium and calculation of the "Most Probable Number" (MPN) of organisms by reference to MPN tables. This method can be applied to all types of water, including those containing an appreciable amount of suspended matter and high background counts of heterotrophic bacteria. However, it must not be used for the enumeration of coliform bacteria in marine water. When using for the enumeration of *E. coli* in marine waters, a 1→10 dilution in sterile water is typically required, although the method has been shown to work well with some marine waters that have a lower than normal concentration of salts. In the absence of data to support the use of the method without dilution, a 1→10 dilution is used.

This method relies upon the detection of *E. coli* based upon expression of the enzyme β-D-glucuronidase and consequently does not detect many of the enterohaemorhagic strains of *E. coli*, which do not typically express this enzyme. Additionally, there are a small number of other *E. coli* strains that do not express β-D-glucuronidase.

The choice of tests used in the detection and confirmation of the coliform group of bacteria, including *E. coli*, can be regarded as part of a continuous sequence. The extent of confirmation with a particular sample depends partly on the nature of the water and partly on the reasons for the examination. The test described in this part of ISO 9308 provides a confirmed result with no requirement for further confirmation of positive wells.

NOTE While this method describes the use of an enumeration device that is commercially available, the medium described here can also be used in a standard MPN format.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 8199, *Water quality — General guide to the enumeration of micro-organisms by culture*

ISO/IEC Guide 2:2004, *Standardization and related activities — General vocabulary*

