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Standards

Irish Standard  
I.S. EN ISO 9308-2:2014

# Water quality - Enumeration of Escherichia coli and coliform bacteria - Part 2: Most probable number method (ISO 9308-2:2012)

## I.S. EN ISO 9308-2:2014

*Incorporating amendments/corrigenda/National Annexes issued since publication:*

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*This document is based on:*

EN ISO 9308-2:2014

*Published:*

2014-04-16

*This document was published under the authority of the NSAI and comes into effect on:*

2014-04-29

ICS number:

07.100.20

NOTE: If blank see CEN/CENELEC cover page

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EUROPEAN STANDARD

EN ISO 9308-2

NORME EUROPÉENNE

EUROPÄISCHE NORM

April 2014

ICS 07.100.20

English Version

## Water quality - Enumeration of Escherichia coli and coliform bacteria - Part 2: Most probable number method (ISO 9308-2:2012)

Qualité de l'eau - Dénombrement des Escherichia coli et des bactéries coliformes - Partie 2: Méthode du nombre le plus probable (ISO 9308-2:2012)

Wasserbeschaffenheit - Zählung von Escherichia coli und coliformen Bakterien - Teil 2: Verfahren zur Bestimmung der wahrscheinlichsten Keimzahl (ISO 9308-2:2012)

This European Standard was approved by CEN on 11 April 2014.

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## **Foreword**

The text of ISO 9308-2:2012 has been prepared by Technical Committee ISO/TC 147 "Water quality" of the International Organization for Standardization (ISO) and has been taken over as EN ISO 9308-2:2014 by Technical Committee CEN/TC 230 "Water analysis" the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by October 2014, and conflicting national standards shall be withdrawn at the latest by October 2014.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

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### **Endorsement notice**

The text of ISO 9308-2:2012 has been approved by CEN as EN ISO 9308-2:2014 without any modification.

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# INTERNATIONAL STANDARD

**ISO**  
**9308-2**

Second edition  
2012-07-01

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## **Water quality — Enumeration of *Escherichia coli* and coliform bacteria —**

Part 2:

### **Most probable number method**

*Qualité de l'eau — Dénombrement des *Escherichia coli* et des  
organismes coliformes —*

*Partie 2: Méthode du nombre le plus probable*



Reference number  
ISO 9308-2:2012(E)

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Published in Switzerland



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## ISO 9308-2:2012(E)

### Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

ISO 9308-2 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 4, *Microbiological methods*.

This second edition cancels and replaces the first edition (ISO 9308-2:1990), which has been technically revised.

ISO 9308 consists of the following parts, under the general title *Water quality — Enumeration of Escherichia coli and coliform bacteria*:

- *Part 1: Membrane filtration method for waters with low bacterial background flora*
- *Part 2: Most probable number method*
- *Part 3: Miniaturized method (Most Probable Number) for the detection and enumeration of E. coli in surface and waste water*

## 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.

ISO takes no position concerning the evidence, validity and scope of these patent rights.

The holder of this patent right has assured the ISO that he/she is willing to negotiate licences either free of charge or under reasonable and non-discriminatory terms and conditions with applicants throughout the world. In this respect, the statement of the holder of this patent right is registered with ISO. Information may be obtained from:

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ISO (<http://www.iso.org/patents>) and IEC (<http://patents.iec.ch>) maintain on-line databases of patents relevant to their standards. Users are encouraged to consult the databases for the most up to date information concerning patents.



# Water quality — Enumeration of *Escherichia coli* and coliform bacteria —

## Part 2: Most probable number method

**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  $\beta$ -D-glucuronidase and consequently does not detect many of the enterohaemorrhagic 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  $\beta$ -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*

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