SLOVENSKI STANDARD
SIST EN ISO 9308-2:2014

01-julij-2014

Nadomešča:
SIST ISO 9308-2:1998

Kakovost vode - Ugotavljanje števila Escherichia coli in koliformnih bakterij - 2. del: Metoda najverjetnejšega števila (ISO 9308-2:2012)

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

Wasserbeschaffenheit - Zählung von Escherichia coli und coliformen Organismen - Teil 2: Verfahren zur Bestimmung der Anzahl mit der höchsten Wahrscheinlichkeit (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)

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ICS:

07.100.20	Mikrobiologija vode	Microbiology of water
13.060.01	Kakovost vode na splošno	Water quality in general

SIST EN ISO 9308-2:2014 **en**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN ISO 9308-2

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)

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COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

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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of an identical text or by endorsement, at the latest by October 2014, and conflicting national standards shall be withdrawn at the latest by October 2014.

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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.

INTERNATIONAL STANDARD

ISO 9308-2

Second edition
2012-07-01

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

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Foreword

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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.

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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 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*

ISO 19458, *Water quality — Sampling for microbiological analysis*

3 Terms and definitions

For the purpose of this document, the terms and definitions given in ISO/IEC Guide 2 and the following apply.

3.1
coliform bacterium
member of the *Enterobacteriaceae* that express the enzyme β-D-galactosidase

3.2
Escherichia coli
member of the *Enterobacteriaceae* that expresses both β-D-galactosidase and β-D-glucuronidase enzymes

4 Principle

A snap pack of dehydrated medium is added to a sample of water (100 ml), or a dilution of a sample made up to 100 ml. Sample plus medium is gently shaken to ensure adequate mixing and to afford dissolution of the medium. The sample plus medium is then aseptically poured into a Quanti-Tray[1)] or Quanti-Tray/2000[1)] to enumerate up to 201 organisms or 2 419 organisms per 100 ml, respectively. Trays are sealed with a Quanti-Tray[1)] Sealer and then incubated at (36 ± 2) °C for 18 h to 22 h.

After incubation, sample wells that have a yellow colour of equal or greater intensity than that of the comparator wells are considered positive for coliform bacteria. Yellow wells that also exhibit any degree of fluorescence are considered positive for *E. coli*.

By means of statistical tables, or a simple computer program, the most probable number (MPN) of coliform bacteria and *E. coli* in 100 ml of the sample can be determined.

NOTE The yellow colouration can be seen with the naked eye and results from the cleavage of ortho-nitrophenol galactoside by the enzyme β-D-galactosidase. The fluorescence is demonstrable under ultraviolet light (365 nm) and originates from the cleavage of the molecule 4-methylumbelliferyl glucuronide (MUG) by the enzyme β-D-glucuronidase to produce the fluorescent compound methyl umbelliferone.

5 Apparatus and glassware

Use microbiological laboratory equipment and, in particular, the following:

5.1 Apparatus for sterilization by steam (autoclave)

Apparatus and glassware not supplied sterile shall be sterilized according to the instructions given in ISO 8199.

5.2 Hot air oven, for dry heat sterilization.

5.3 Incubator, thermostatically controlled at (36 ± 2) °C.

5.4 Quanti-Tray[1)] sealer.

5.5 Sterile wide mouthed vessels of at least 110 ml.

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5.6 Quanti-Tray[2] comparator.

5.7 Ultraviolet lamp, 365 nm.

5.8 Quanti-Tray[2] or Quanti-Tray/2000[2], see Annex B.

6 Culture media and reagents

6.1 Basic materials

The method utilises Colilert[3]-18 a medium based on the Defined Substrate Technology available for a 100 ml sample as a ready to use powder dispensed in snap packs. Each snap pack contains sufficient medium (2,8 g) for a single test. Medium is stored under ambient conditions (2 °C to 25 °C) out of direct sunlight and should be used before the expiry date listed on the snap pack.

The medium is composed of two components to give the final concentrations as shown in Annex C.

6.2 Diluent

For dilutions to be used with Colilert[3]-18, use only sterile, non-inhibitory, oxidant-free water (deionized or tap). The use of buffered, saline or peptone-containing diluents interferes with the performance of the test.

6.3 Antifoam B

Antifoam B is a 10 % active, water soluble suspension of silicone.

7 Sampling

Take the samples and deliver them to the laboratory in accordance with ISO 19458.

8 Procedure

8.1 Preparation of the sample

Samples should be transported and stored at (5 ± 3) °C in accordance with ISO 19458 and analysis commenced on the day of collection or within 18 h. Under exceptional circumstances, the samples may be kept at (5 ± 3) °C for up to 24 h prior to examination.

8.2 Inoculation of media

Aseptically add a single snap pack of Colilert[4]-18 medium (2,8 g) to each 100 ml volume of sample or dilution. When the medium has completely dissolved, the sample plus medium is aseptically poured into either a

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