INTERNATIONAL STANDARD

ISO 9308-1

Second edition
2000-09-15

Water quality — Detection and enumeration of *Escherichia coli* and coliform bacteria —

Part 1: Membrane filtration method

Qualité de l'eau — Recherche et dénombrement des Escherichia coli *et des bactéries coliformes —*

Partie 1: Méthode par filtration sur membrane

Reference number
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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 3.

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.

Attention is drawn to the possibility that some of the elements of this part of ISO 9308 may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 9308-1 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 9308-1:1990), which has been technically revised.

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

— *Part 1: Membrane filtration method*

— *Part 2: Liquid enrichment method*

— *Part 3: Miniaturized method (Most Probable Number, MPN) for detection and enumeration of* E. coli *in surface and waste water*

Annex B forms a normative part of this part of ISO 9308. Annex A is for information only.

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*, 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 failure in treatment or distribution. The identification of the strains isolated can sometimes provide an indication of their origin.

Water quality — Detection and enumeration of *Escherichia coli* and coliform bacteria —

Part 1:
Membrane filtration method

1 Scope

This part of ISO 9308 describes a reference method (Standard Test) for the detection and enumeration of *Escherichia coli* and coliform bacteria in water for human consumption. The Standard Test is based on membrane filtration, subsequent culture on a differential agar medium and calculation of the number of target organisms in the sample.

The Standard Test has a low selectivity, allowing the detection of injured bacteria. Due to the low selectivity, background growth can interfere with the reliable enumeration of coliform bacteria and *E. coli*, for example in some drinking waters, like shallow well waters, that have not been disinfected and yield a high background growth. This part of ISO 9308 is therefore especially suitable for disinfected water and other drinking waters of low bacterial numbers.

This part of ISO 9308 includes a rapid method (Rapid Test) for the detection of *E. coli* only within 24 h in water for human consumption, which can be useful in special cases when information is needed quickly. The Rapid Test is based on membrane filtration, subsequent culture under selective conditions and calculation of the number of *E. coli* in the sample.

Standard and Rapid Tests described in this part of ISO 9308 are applicable to other kinds of water provided that suspended matter or background flora does not interfere with filtration, culture and counting.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this part of ISO 9308. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this part of ISO 9308 are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

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

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods.*

ISO 5667-1:1980, *Water quality — Sampling — Part 1: Guidance on the design of sampling programmes.*

ISO 5667-2:1991, *Water quality — Sampling — Part 2: Guidance on sampling techniques.*

ISO 5667-3:1994, *Water quality — Sampling — Part 3: Guidance on the preservation and handling of samples.*

ISO 6887-1:1999, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions.*

ISO 8199:1988, *Water quality — General guide to the enumeration of micro-organisms by culture.*

3 Terms and definitions

For the purposes of this part of ISO 9308, the terms and definitions given in ISO/IEC Guide 2 and the following apply.

3.1
lactose-positive bacteria
⟨Standard Test⟩ bacteria capable of forming colonies aerobically at (36 ± 3) °C on a selective and differential lactose culture medium with the production of acid within (21 ± 3) h

3.2
coliform bacteria
⟨Standard Test⟩ lactose-positive bacteria as defined in 3.1 which are oxidase-negative

3.3
Escherichia coli
⟨Standard Test⟩ coliform bacteria as defined in 3.2 which also produce indole from tryptophan at (44,0 ± 0,5) °C within (21 ± 3) h

3.4
Escherichia coli
⟨Rapid Test⟩ bile-resistant bacteria which also produce indole from tryptophan at (44,0 ± 0,5) °C within (21 ± 3) h

4 Principle

4.1 General description of the method

The method is based on membrane filtration and consists of two parts, the reference Standard Test and the optional Rapid Test, which can be performed in parallel as described below. The Standard Test includes incubation of the membrane on a selective medium with subsequent further biochemical characterization of the typical lactose-positive colonies, leading to the detection and enumeration of coliform bacteria and *E. coli* within 2 d to 3 d. The Rapid Test consists of two incubation steps allowing the detection and enumeration of *E. coli* within (21 ± 3) h. If both tests, Standard Test and Rapid Test, are performed in parallel, the final result for *E. coli* shall be the higher of the two.

4.2 Filtration and incubation

Test portions of the sample are filtered through membranes which retain the bacteria. One membrane (Standard Test) is placed on a selective lactose agar medium which is incubated at (36 ± 2) °C for (21 ± 3) h and one membrane (Rapid Test) on a casein (tryptic digest)-containing agar medium incubated at (36 ± 2) °C for 4 h to 5 h, followed by incubation at (44,0 ± 0,5) °C for 19 h to 20 h on an agar medium containing casein (tryptic digest) and bilesalts.

4.3 Evaluation and confirmation, Standard Test

The characteristic colonies on the membrane are counted as lactose-positive bacteria. For coliform bacteria and *E. coli*, subculture is carried out of randomly selected characteristic colonies for confirmatory tests: oxidase and indole production. The numbers of lactose-positive coliform bacteria and *E. coli* likely to be present in 100 ml of the sample are counted.

4.4 Evaluation and confirmation, Rapid Test

The colonies on the membrane which are able to form indole from the tryptophan supplied in the agar medium are counted as *E. coli*. The numbers of *E. coli* likely to be present in 100 ml of the sample are counted.

5 Apparatus and glassware

Usual microbiological laboratory equipment, and in particular:

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 Water bath and/or incubator, thermostatically controlled at (36 ± 2) °C.

5.3 Water bath and/or incubator, thermostatically controlled at (44,0 ± 0,5) °C.

NOTE For the Rapid Test, instead of the incubators 5.2 and 5.3 a programmable incubator with dual setting may be used, set to (36 ± 2) °C and (44,0 ± 0,5) °C.

5.4 pH meter, with an accuracy of ± 0,1.

5.5 Equipment for membrane filtration, in accordance with ISO 8199.

5.6 Membrane filters, composed of cellulose esters, usually about 47 mm or 50 mm in diameter, with filtration characteristics equivalent to a rated nominal pore diameter of 0,45 µm and preferably with grids.

The filters shall be free from growth-inhibiting or growth-promoting properties and the printing ink used for the grid shall not affect the growth of bacteria. If not obtained sterile, they shall be sterilized in accordance with the manufacturer's instructions. Every batch of membranes should be tested in accordance with ISO 7704 for its suitability for the test, especially since the use of different brands of filter may result in a difference in colour development.

NOTE Green membrane filters may be helpful when using the Rapid Test for a better detection of colour development.

5.7 Forceps with rounded tips for handling membranes.

5.8 Ultraviolet lamp, wavelength 254 nm (low-pressure mercury lamp).

WARNING — UV light causes irritation of eyes and skin. Use protective glasses and gloves.

5.9 Filter pads, with a diameter of at least 47 mm.

6 Culture media and reagents

For the preparation of culture media and reagents, use ingredients of uniform quality and chemicals of analytical grade (see note); follow the instructions given in annex B. Alternatively, use commercially available media and reagents which comply with the compositions given in annex B and follow strictly the manufacturer's instructions.

NOTE The use of chemicals of other grade is possible, providing they are shown to be of equal performance in the test.

For preparation of culture media, use glass-distilled water or deionized water free from substances which might inhibit bacterial growth under the conditions of the test, and which is in accordance with ISO 3696.

Unless specified otherwise, the prepared media are stable for at least one month if stored in the dark at (5 ± 3) °C and protected against evaporation.