



SLOVENSKI STANDARD SIST EN ISO 9308-3:1999

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Water quality - Detection and enumeration of Escherichia coli and coliform bacteria in surface and wastewater - Part 3: Miniaturized method (Most Probable Number) by inoculation in liquid medium (ISO 9308-3:1998)

Wasserbeschaffenheit - Nachweis und Zählung von Escherichia coli und coliformen Bakterien in Oberflächenwasser und Abwasser - Teil 3: Miniaturisiertes Verfahren durch Animpfen in Flüssigmedium (MPN-Verfahren) (ISO 9308-3:1998)

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Qualité de l'eau - Recherche et dénombrement des Escherichia coli et des bactéries coliformes dans les eaux de surface et résiduares - Partie 3: Méthode miniaturisée (nombre le plus probable) pour ensemencement en milieu liquide (ISO 9308-3:1998)

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English version

Water quality - Detection and enumeration of Escherichia coli
and coliform bacteria in surface and waste water - Part 3:
Miniaturized method (Most Probable Number) by inoculation in
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liquide (ISO 9308-3:1998)

This European Standard was approved by CEN on 15 November 1998.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and United Kingdom.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

Central Secretariat: rue de Stassart, 36 B-1050 Brussels

Foreword

The text of the International Standard ISO 9308-3:1998 has been prepared by Technical Committee ISO/TC 147 "Water quality" in collaboration with 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 May 1999, and conflicting national standards shall be withdrawn at the latest by May 1999.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

NOTE FROM CEN/CS: The foreword is susceptible to be amended on reception of the German language version. The confirmed or amended foreword, and when appropriate, the normative annex ZA for the references to international publications with their relevant European publications will be circulated with the German version.

Endorsement notice

The text of the International Standard ISO 9308-3:1998 was approved by CEN as a European Standard without any modification.

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**Water quality — Detection and enumeration
of *Escherichia coli* and coliform bacteria
in surface and waste water —**

Part 3:

Miniaturized method (Most Probable Number)
by inoculation in liquid medium

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*Qualité de l'eau — Recherche et dénombrement des Escherichia coli
et des bactéries coliformes dans les eaux de surface et résiduaires —*

*Partie 3: Méthode miniaturisée (nombre le plus probable) pour
ensemencement en milieu liquide*

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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.

International Standard ISO 9308-3 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 4, *Microbiological methods*.

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

- Part 1: *Membrane filtration method*
- Part 2: *Liquid enrichment method*
- Part 3: *Miniaturized method (Most Probable Number) by inoculation in liquid media*

Annexes E and F form a normative part of this part of ISO 9308. Annexes A to D are for information only.

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Water quality — Detection and enumeration of *Escherichia coli* and coliform bacteria in surface and waste water —

Part 3: Miniaturized method (Most Probable Number) by inoculation in liquid medium

1 Scope

This part of ISO 9308 specifies a miniaturized method for the detection and enumeration of *Escherichia coli* (*E. coli*) in surface and waste water by inoculation in a liquid medium. The method is applicable to all types of surface and waste waters, particularly those rich in suspended matter.

This method is not suitable for drinking water and any other type of water for which the guideline is less than 15 counts per 100 ml.

This method is not appropriate for enumeration and detection of coliform bacteria other than *E. coli*.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard 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 3951, *Sampling procedures and charts for inspection by variables for percent nonconforming*.

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

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

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

ISO 8199, *Water quality — General guide to the enumeration of microorganisms by culture*.

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

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

Escherichia coli

E. coli

β -D-glucuronidase-positive microorganism growing at an incubation temperature of 44 °C in the specified liquid medium containing 4-methylumbelliferyl- β -D-glucuronide (MUG)

4 Principle

The diluted sample is inoculated in a row of microtitre plate wells containing dehydrated culture medium.

The microtitre plates are examined under ultraviolet light at 366 nm in the dark after an incubation period of 36 h minimum and 72 h maximum at 44 °C \pm 0,5 °C. The presence of *E. coli* is indicated by a blue fluorescence resulting from hydrolysis of MUG. The results are given as most probable number (MPN) per 100 ml.

5 Apparatus

With the exception of equipment supplied sterile, the glassware shall be sterilized in accordance with the instructions given in ISO 8199.

Usual microbiological laboratory equipment, and in particular:

5.1 Apparatus for sterilization by dry heat (oven) or steam (autoclave).

5.2 **Thermostatic incubator** regulated at 44 °C \pm 0,5 °C.

5.3 Tunnel drier or vertical laminar air flow cabinet (preferably class II).

5.4 **UV observation chamber** (Wood's Lamp, 366 nm).

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

5.5 Portable refractometer (optional).

5.6 **pH meter** with an accuracy of \pm 0,1

5.7 **Test tubes** of dimensions 16 mm \times 160 mm and 20 mm \times 200 mm, or flasks with similar capacity.

5.8 **8-channel multipipette**, adjustable or preset, or any other system suitable for measuring and distributing 200 μ l per well.

5.9 **Sterile tips** for multipipette.

5.10 **Equipment for membrane filtration** in accordance with ISO 8199, including membrane filters with a nominal pore size of 0,2 μ m, for sterilization of liquid media.

5.11 **Sterile microtitre plates**, 96-well, 350 μ l, flat bottomed, nonfluorescent.

5.12 Sterile adhesive covering strips for sealing microtitre plates.

5.13 **Sterile Petri dishes**, 90 mm in diameter.

6 Sampling

Take the samples and deliver them to the laboratory in accordance with ISO 8199 and ISO 5667-1, ISO 5667-2 and ISO 5667-3.

7 Culture media and diluent

7.1 General instructions

To ensure reproducible results, prepare culture medium and diluents, using either constituents of uniform quality and chemicals of recognized analytical grade, or a dehydrated diluent or complete medium prepared following the manufacturer's instructions. Prepare them with demineralized or distilled water free from substances capable of inhibiting growth under the test conditions. If the media are not used immediately, preserve them in the dark at $(5 \pm 3) ^\circ\text{C}$ for up to one month in conditions avoiding any alterations to their composition.

NOTE The use of chemicals of other grades is permissible providing they are shown to be of equivalent performance in the test.

7.2 Diluents

7.2.1 Special diluent (SD)

Synthetic sea salt ¹⁾	22,5 g
Bromophenol blue solution (optional)	10 ml
Demineralized or distilled water (7.2.2)	1000 ml

Sterilize in the autoclave (5.1) at $121 ^\circ\text{C} \pm 3 ^\circ\text{C}$ for 15 min to 20 min.

The bromophenol blue solution is prepared by adding 0,04 g in 100 ml of 50 % ethanol. It is only used to colour the SD blue and avoid confusing it with demineralized or distilled water.

7.2.2 Demineralized or distilled water, free from substances inhibiting growth under the test conditions.

Sterilize in the autoclave (5.1) at $121 ^\circ\text{C} \pm 3 ^\circ\text{C}$ for 15 min to 20 min.

7.3 Culture medium: MUG/EC medium

Composition

Tryptone	40 g
Salicin	1 g
Triton X 100®	1 g
MUG (4-methylumbelliferyl- β -D-glucuronide)	100 mg
Demineralized or distilled water (7.2.2)	1000 ml

¹⁾ A typical analysis of a commercially available and suitable synthetic sea salt is given in annex C. Other diluents, such as distilled water, can be used for *E. coli* enumeration, unless intestinal enterococci are to be enumerated from the same dilution tubes.