SLOVENSKI STANDARD

SIST EN ISO 7899-1:1999

01-november-1999

Kakovost vode ï Ugotavljanje prisotnosti in ģtevila intestinalnih enterokokov v povrģinskih in odpadnih vodah ï 1. del: Metoda z miniaturizacijo (najbolj verjetno ģtevilo) z nasajanjem v tekoļ em gojiģļ u (ISO 7899-1:1998)

Water quality - Detection and enumeration of intestinal enterococci in surface and wastewater - Part 1: Miniaturized method (Most Probable Number) by inoculation in liquid medium (ISO 7899-1:1998)

Wasserbeschaffenheit - Nachweis und Zählung von intestinalen Enterokokken in Oberflächenwasser und Abwasser - Teil 1: Miniaturisiertes Verfahren durch Animpfen in Flüssigmedium (MPN-Verfahren) (ISO 7899-1:1998)

Qualité de l'eau - Recherche et dénombrement des entérocoques intestinaux dans les eaux de surface et résiduaires - Partie 1: Méthode miniaturisée (nombre le plus probable) par ensemencement en milieu liquide (ISO 7899-1:1998)

Ta slovenski standard je istoveten z: EN ISO 7899-1:1998

ICS:

07.100.20	Mikrobiologija vode	Microbiology of water

SIST EN ISO 7899-1:1999 en

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN ISO 7899-1

November 1998

ICS 07.100.20

Descriptors: see ISO document

English version

Water quality - Detection and enumeration of intestinal enterococci in surface and waste water - Part 1: Miniaturized method (Most Probable Number) by inoculation in liquid medium (ISO 7899-1:1998)

Qualité de l'eau - Recherche et dénombrement des entérocoques intestinaux dans les eaux de surface et résiduaires - Partie 1: Méthode miniaturisée (nombre le plus probable) par ensemencement en milieu liquide (ISO 7899-1:1998)

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.

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 7899-1:1998 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 7899-1:1998 was approved by CEN as a European Standard without any modification.

INTERNATIONAL STANDARD

ISO 7899-1

Second edition
1998-11-15

Water quality — Detection and enumeration of intestinal enterococci in surface and waste water —

Part 1:
Miniaturized method (Most Probable Number) by inoculation in liquid medium

Qualité de l'eau — Recherche et dénombrement des entérocoques intestinaux dans les eaux de surface et résiduaires —

Partie 1: Méthode miniaturisée (nombre le plus probable) par ensemencement en milieu liquide

Reference number
ISO 7899-1:1998(E)

Contents

International Organization for Standardization
Case postale 56 • CH-1211 Genève 20 • Switzerland
Internet iso@iso.ch

Printed in Switzerland

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.

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 7899-1 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 4, *Biological methods.*

This second edition cancels and replaces the first edition (ISO 7899-1:1984), which has been technically revised.

ISO 7899 consists of the following parts, under the general title *Water quality — Detection and enumeration of intestinal enterococci in surface and waste water*:

— *Part 1: Miniaturized method (Most Probable Number) by inoculation in liquid medium*

— *Part 2: Method by membrane filtration*

Annexes E and F form an integral part of this part of ISO 7899. Annexes A, B, C, D and G are for information only.

Introduction

The aim of this part of ISO 7899 is to enumerate the major intestinal enterococci, namely *E. faecalis*, *E. faecium*, *E. durans* and *E. hirae*, which occur frequently in faeces of humans and homeothermic animals. Other faecal *Enterococcus* species, namely *E. avium*, *E. cecorum*, *E. columbae* and *E. gallinarum*, and *Streptococcus bovis*/*equinus* strains may occasionally be included, but they occur rarely in the environmental samples. Their recovery tends to be low. *Enterococcus casseliflavus* and *E. mundtii* are non-faecal species which, when present in water samples (e.g. because of influence of plant material and some industrial effluents), are enumerated as faecal enterococci. These species and other rare non-faecal species tend to produce yellow pigment on a non-selective medium. The possible interference of non-faecal *Enterococcus* species should therefore be considered in the interpretation of results.

Water quality — Detection and enumeration of intestinal enterococci in surface and waste water —

Part 1:
Miniaturized method (Most Probable Number) by inoculation in liquid medium

1 Scope

This part of ISO 7899 specifies a miniaturized method for the detection and enumeration of major intestinal enterococci 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 count is less than 15 per 100 ml.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this part of ISO 7899. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this part of ISO 7899 are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 3951:1989, *Sampling procedures and charts for inspection by variables for percent nonconforming*.

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 8199:1988, *Water quality — General guide to the enumeration of microorganisms by culture*.

ISO/IEC Guide 2:1996, *Standardization and related activities — Vocabulary.*

3 Definitions

For the purposes of this part of ISO 7899, the definitions given in ISO/IEC Guide 2 and the following definition apply.

3.1
intestinal enterococci
microorganisms capable of aerobic growth at 44 °C and of hydrolysing the 4-methylumbelliferyl-β-D-glucoside (MUD), in the presence of thallium acetate, nalidixic acid and 2,3,5-triphenyltetrazolium chloride (TTC), in the liquid medium specified

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 between 36 h and 72 h at 44 °C ± 0,5 °C. The presence of enterococci is indicated by fluorescence resulting from the hydrolysis of MUD. 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 by steam (autoclave).

5.2 Thermostatic incubator, regulated at 44 °C ± 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 can cause irritation of skin and eyes. Use protective gloves and glasses.

5.5 Portable refractometer (optional).

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

5.7 Test tubes, 16 mm x 160 mm and 20 mm x 200 mm**, or flasks** with similar capacity.

5.8 Adjustable or pre-set 8-channel multipipette, or any system suitable for measuring and distributing 200 µl 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 µm, for sterilization of liquid media.

5.11 Sterile microtitre plates, 96-well, 350 µl, flat-bottomed, nonfluorescent.

5.12 Sterile adhesive cover 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 diluents

7.1 General instructions

To ensure reproducible results, prepare culture medium and diluents, using either constituents of uniform quality and chemicals of recognized analytical or a dehydrated diluent or complete medium prepared following the manufacturer's instructions. Prepare them with distilled or demineralized water, free from substances capable of inhibiting or promoting growth under the test conditions. If the media are not used immediately, preserve them in the dark at (5 ± 3) °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 Diluent

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 °C ± 3 °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 used only to colour the SD blue and avoid confusing it with demineralized or distilled water.

7.2.2 Demineralized or distilled water

Water used for dilution shall be demineralized or distilled water free from substances inhibiting growth under the test conditions.

Sterilize in the autoclave (5.1) before use at 121 °C ± 3 °C for 15 min to 20 min.

7.3 Culture medium: MUD/SF medium

7.3.1 Composition

7.3.1.1 Solution A

Tryptose	40 g
KH_2PO_4	10 g
D(+)-galactose	2 g
Polyoxyethylenesorbitan monooleate (Tween® 80[2)])	1,5 ml
Demineralized or distilled water (7.2.2)	900 ml

Add tryptose, KH_2PO_4, galactose and Tween® 80 to 900 ml of water, whilst maintaining gentle heat and magnetic stirring, then bring to the boil until completely dissolved. Allow to cool.

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1) A typical analysis of a commercially available and suitable synthetic sea salt is given in annex C. Pure NaCl solutions are not suitable, as they lead to marked inhibition.

2) Tween® 80 is an example of a suitable product available commercially. This information is given for the convenience of users of this part of ISO 7899 and does not constitute an endorsement by ISO of this product.