SLOVENSKI STANDARD
SIST EN ISO 8199:2007

01-december-2007

Nadomešča:
SIST ISO 8199:1997

Kakovost vode - Splošno navodilo za štetje mikroorganizmov v gojišču (ISO 8199:2005)

Water quality - General guidance on the enumeration of micro-organisms by culture (ISO 8199:2005)

Wasserbeschaffenheit - Allgemeine Anleitung zur Zählung von Mikroorganismen durch Kulturverfahren (ISO 8199:2005)

Qualité de l'eau - Lignes directrices générales pour le dénombrement des micro-organismes sur milieu de culture (ISO 8199:2005)

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ICS:

07.100.20	Mikrobiologija vode	Microbiology of water

SIST EN ISO 8199:2007 **en**

EUROPEAN STANDARD

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EUROPÄISCHE NORM

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October 2007

ICS 07.100.20

English Version

Water quality - General guidance on the enumeration of micro-organisms by culture (ISO 8199:2005)

Qualité de l'eau - Lignes directrices générales pour le dénombrement des micro-organismes sur milieu de culture (ISO 8199:2005)

Wasserbeschaffenheit - Allgemeine Anleitung zur Zählung von Mikroorganismen durch Kulturverfahren (ISO 8199:2005)

This European Standard was approved by CEN on 27 September 2007.

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

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Foreword

The text of ISO 8199:2005 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 8199:2007 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of an identical text or by endorsement, at the latest by April 2008, and conflicting national standards shall be withdrawn at the latest by April 2008.

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

Endorsement notice

The text of ISO 8199:2005 has been approved by CEN as a EN ISO 8199:2007 without any modification.

INTERNATIONAL STANDARD

ISO 8199

Second edition
2005-06-15

Water quality — General guidance on the enumeration of micro-organisms by culture

Qualité de l'eau — Lignes directrices générales pour le dénombrement des micro-organismes sur milieu de culture

Reference number
ISO 8199:2005(E)

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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 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.

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

ISO 8199 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 8199:1988), which has been technically revised.

Introduction

Techniques for the isolation and enumeration of micro-organisms, based on their ability to grow on specified culture media, are an important and widely used means of assessing the microbiological quality of water. The purpose of this guide is to gather in a single document the information common to the various enumeration techniques so as to avoid repetition of technical details in individual standards and to facilitate the choice of the technique most suitable for a particular problem.

Water quality — General guidance on the enumeration of micro-organisms by culture

WARNING — Persons using this International Standard should be familiar with normal laboratory practice. This 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 International Standard be carried out by suitably trained staff.

1 Scope

This International Standard presents guidance for carrying out manipulations which are common to each technique for the microbiological examination of water, particularly the preparation of samples, culture media and apparatus. It also describes the various enumeration techniques available and the criteria for the choice of a particular technique. This International Standard is mainly intended for bacteria, yeasts and moulds. Some aspects are also applicable to viruses and parasites.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

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

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

3 Principle

The general principle of these techniques consists of inoculating a known volume of a water sample on or into a culture medium (solid or liquid). It is assumed that after incubation each micro-organism present multiplies, giving either a colony visible directly on the solid medium, or changes in observable properties of the liquid medium. The choice of a particular method depends not only on the nature of the micro-organisms sought, but also on the nature of the water and the reasons for the examination.

4 General

Uniformity of temperatures and (incubation) times: The following accepted ranges of temperatures and times during incubation or storage are applied, when appropriate for the intended target organism, and unless otherwise stated in the specific standard.

Storage temperatures:	(– 70 ± 10) °C; (– 20 ± 5) °C; (5 ± 3) °C;
Incubation temperatures:	(22 ± 2) °C; (36 ± 2) °C;
Sterilization temperatures:	(115 ± 3) °C; (121 ± 3) °C; (170 ± 10) °C;
Incubation times:	(21 ± 3) h; (44 ± 4) h; (68 ± 4) h.

Other times and temperatures may be specified for specific methods when necessary.

The upper incubation temperature limits are very strict (they can have large influences on the growth). The lower temperature limits may be exceeded for short periods, e.g. due to opening the door of an incubator, but recovery shall be rapid.

Tolerances on volumes and masses: Unless otherwise stated, the accepted range of any measured value is: stated value ± 5 %.

5 Diluents and culture media

5.1 General

5.1.1 Quality requirements

Use constituents of uniform quality and analytical-grade chemicals for the preparation of media. Other grades of chemical may be used provided they can be shown to produce the same results. Alternatively, dehydrated complete media or diluents may be used. Follow the manufacturer's instructions strictly.

Use glass-distilled or demineralized water which is free from substances that might affect growth of micro-organisms under the test conditions. The water shall comply with the requirements of ISO 3696:1987, grade 3.

Unless otherwise stated, the ingredients are added to the volume of water instead of making up to a certain volume, as is normal in the preparation of microbiological culture media.

Before use, check the quality of the media, diluents and filters, e.g. by following the procedures described in ISO 7704, ISO/TS 11133-1 or ISO/TS 11133-2.

5.1.2 Sterilization

Dispense diluents and culture media in containers suitable for sterilization by autoclaving. For most purposes, a temperature of (121 ± 3) °C for 15 min is adequate. However, a different time and temperature may sometimes be required and details are given in each individual standard.

Alternatively, with thermolabile substances, removal of micro-organisms may be effected by filtration through a filter with a pore size of 0,2 µm, specified by the manufacturer as being suitable for “sterilization”.

5.2 Diluents

The diluents given in this subclause are commonly used in water microbiology. However, the list is not exhaustive and other appropriate diluents may be used.

After preparation, distribute each solution into bottles and sterilize, e.g. by autoclaving at (121 ± 3) °C for 15 min. Alternatively, the diluent can be aseptically distributed after sterilization. Store at room temperature or in a refrigerator at (5 ± 3) °C for a maximum of 6 months. If a diluent shows any change from its normal appearance, discard it.

5.2.1 Saline solution

Composition

Sodium chloride (NaCl)	8,5 g
Water (see 5.1.1)	1 000 ml

Preparation

Dissolve the ingredients in the water, if necessary by heating. Adjust the pH by adding sodium hydroxide solution [c(NaOH) = 1 mol/l] or hydrochloric acid [c(HCl) = 1 mol/l] so that, after sterilization (see 5.1.2), it will correspond to 7,0 ± 0,5 at 25 °C.

5.2.2 Peptone diluent

Composition

Enzymatic digest of casein (peptone)	1,0 g
Water (see 5.1.1)	1 000 ml

Preparation

Dissolve the ingredients in the water, if necessary by heating. Adjust the pH by adding sodium hydroxide solution [c(NaOH) = 1 mol/l] or hydrochloric acid [c(HCl) = 1 mol/l] so that, after sterilization (see 5.1.2), it will correspond to 7,0 ± 0,5 at 25 °C.

5.2.3 Peptone saline solution

Composition

Enzymatic digest of casein (peptone)	1,0 g
Sodium chloride (NaCl)	8,5 g
Water (see 5.1.1)	1 000 ml

Preparation

Dissolve the ingredients in the water, if necessary by heating. Adjust the pH by adding sodium hydroxide solution [c(NaOH) = 1 mol/l] or hydrochloric acid [c(HCl) = 1 mol/l] so that, after sterilization (see 5.1.2), it will correspond to 7,0 ± 0,5 at 25 °C.

5.2.4 Ringer's solution, quarter-strength

Composition

Sodium chloride (NaCl)	2,25 g
Potassium chloride (KCl)	0,105 g
Calcium chloride (anhydrous) ($CaCl_2$)	0,12 g
Sodium hydrogen carbonate ($NaHCO_3$)	0,05 g
Water (see 5.1.1)	1 000 ml