SLOVENSKI STANDARD
oSIST prEN ISO 8199:2017

01-november-2017

Kakovost vode - Splošne zahteve in navodilo za štetje mikroorganizmov v gojišču (ISO/DIS 8199:2017)

Water quality - General requirements and guidance for microbiological examinations by culture (ISO/DIS 8199:2017)

Wasserbeschaffenheit - Allgemeine Anleitung zur Zählung von Mikroorganismen durch Kulturverfahren (ISO/DIS 8199:2017)

Qualité de l'eau - Lignes directrices générales pour le dénombrement des micro-organismes sur milieu de culture (ISO/DIS 8199:2017)

Ta slovenski standard je istoveten z: **prEN ISO 8199**

ICS:

07.100.20	Mikrobiologija vode	Microbiology of water

oSIST prEN ISO 8199:2017 **en,fr,de**

DRAFT INTERNATIONAL STANDARD

ISO/DIS 8199

ISO/TC **147**/SC **4** Secretariat: **DIN**

Voting begins on: **2017-10-04**

Voting terminates on: **2017-12-27**

Water quality — General requirements and guidance for microbiological examinations by culture

Titre manque

ICS: 07.100.20

This document is circulated as received from the committee secretariat.

ISO/CEN PARALLEL PROCESSING

Reference number
ISO/DIS 8199:2017(E)

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Contents

Page

Foreword ... iv
Introduction ... v
1 Scope ... 1
2 Normative references ... 1
3 Terms and definitions ... 1
4 Principle ... 4
5 General measurement requirements ... 4
6 Diluents and culture media ... 5
7 Sterilization and decontamination ... 5
8 Samples and sample handling ... 7
9 Enumeration (quantitative) methods ... 8
10 Detection (qualitative) methods ... 32
11 Performance characteristics of methods ... 33
12 Analytical quality control ... 34
Annex A (informative) **Criteria for the choice of technique** ... 37
Annex B (informative) **Confidence intervals for colony count technique and choice of method of calculation in special cases** ... 42
Annex C (normative) **Counting and calculations with two dishes per dilution** ... 46
Annex D (normative) **Composition, preparation and performance testing of diluents** ... 53
Bibliography ... 57

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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 4, *Microbiological methods*.

This third edition cancels and replaces the second edition (ISO 8199:2005), which has been technically revised.

Introduction

Techniques for the detection and enumeration of microorganisms based on their ability to grow on or in specified culture media are an important and widely used means of assessing the microbiological quality of water. The purpose of this document is to gather in a single document the information common to the various techniques. This reduces repetition of technical details in individual standards and facilitates choice of the technique most suitable for a particular situation. Other guidance has been included on general topics of relevance to these techniques, such as analytical quality control, method performance characteristics and uncertainty of test results.

Water quality — General requirements and guidance for microgiological examinations by culture

WARNING — Persons using this document should be familiar with normal laboratory practice. This document 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.

IMPORTANT — It is absolutely essential that tests conducted according to this document be carried out by suitably trained staff.

1 Scope

This document gives requirements and guidance for performing the manipulations common to each culture technique for the microbiological examination of water, particularly the preparation of samples, culture media, and general apparatus and glassware, unless otherwise required in the specific standard. This document also describes the various techniques available for detection and enumeration by culture and the criteria for determining which technique is appropriate. This document is mainly intended for examinations for bacteria, yeasts and moulds, but some aspects are also applicable to bacteriophages, viruses and parasites. Techniques not based on culturing microorganisms, such as polymerase chain reaction (PCR) methods, are excluded from the scope.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 7704, *Water quality — Requirements for evaluation of membrane filters used for microbiological testing*

ISO 11133, *Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media*

3 Terms and definitions

For the purposes of this document the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at http://www.iso.org./obp

3.1
accuracy
closeness of agreement between a test result and the accepted reference value

[SOURCE: ISO 6107-8:1993, 1[8], modified — NOTE 2 deleted]

3.2
bias
difference between the expectation of the test results and an accepted reference value

[SOURCE: ISO 5725-1:1994, 3.8[5]]

3.3
confirmed count
count corrected for not confirmed presumptive counts by further testing of the presumptive objects

3.4
count
<microbiology> observed number of objects such as colonies or cells determined by direct counting, or most probable number (MPN) estimation based on statistical calculation using the number of positive units or presumptive positive units in a dilution series of a test

[SOURCE: ISO 6107-6:2004, 22[7]]

3.5
detection level
minimum concentration of organisms that produce evidence of growth with a probability of p = 0,95 when inoculated into a specified culture medium and incubated under defined condition

[SOURCE: ISO 13843:2017, 3.10[14], modified — NOTE 1 to entry deleted]

3.6
intra-laboratory reproducibility (intermediate precision)
closeness of agreement between test results obtained with the same method on different test materials in the same laboratory with different operators using different equipment

3.7
limit of determination
lowest average particle concentration per analytical portion where the expected relative standard uncertainty, equals a specified value (relative standard deviation)

[SOURCE: ISO 13843:2017, 3.17[14]]

3.8
precision
closeness of agreement between independent test results obtained under stipulated conditions

[SOURCE: ISO 5725-1:1994, 3.12[5]]

3.9
presumptive count
colony count or most probable number (MPN) estimate based on the number of colonies or reaction vessels that have an outward appearance that is interpreted as typical of a target organism

[SOURCE: ISO 6107-6:2004, 62[7]]

3.10
relative standard deviation
estimate of the standard deviation s of a population from a sample of n results divided by the mean $\bar{x}$ of that sample

[SOURCE: ISO 13843:2017, 3.30[14], modified — "s" and "$\bar{x}$" added]

3.11
repeatability
closeness of agreement between mutually independent test results obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within short intervals of time

3.12
reproducibility
closeness of agreement between test results obtained with the same method on identical test material in different laboratories with different operators using different equipment

3.13
test portion
specified quantity of the sample that is taken for analysis e.g. 0,1 ml, 1 ml, 100 ml of sample

3.14
test sample
undiluted, diluted or otherwise prepared test portion of a sample to be tested, after completion of all preparation steps such as centrifugation, filtration, homogenization, pH adjustment and determination of ionic strength

[SOURCE: ISO 6107-6:2004, 92[7]]

3.15
trueness
closeness of agreement between the average value obtained from a large series of test results and an accepted reference value

Note 1 to entry: The measure of trueness is usually expressed in terms of bias.

[SOURCE: ISO 6107-8:1993, 63[8]]

3.16
uncertainty (of counting)
relative standard deviation of results of repeated counting of the colonies or particles of the same plate(s) or field(s) under stipulated conditions (same person, different persons in one laboratory, or different laboratories)

[SOURCE: ISO 6107-6:2004, 103[7]]

4 Principle

The general principle of these techniques consists of inoculating a test portion of a water sample, or resultant test sample following membrane filtration or centrifugation, on or into a culture medium (solid or liquid). It is assumed that after incubation each target microorganism present multiplies, giving either a colony visible directly on or in the solid medium or changes in the observable properties of the liquid medium. The choice of a particular culture method depends not only on the nature and numbers of the microorganisms sought, but also on the nature of the water and the reasons for the examination.

5 General measurement requirements

5.1 Uniformity of temperatures

The following accepted ranges of temperatures and their ranges for incubation or storage are applied, where appropriate for the intended target organism and unless otherwise required in the specific standard.

Storage temperatures: (−70 ± 10) °C; (−20 ± 5) °C; (5 ± 3) °C

Incubation temperatures: (22 ± 2) °C; (36 ± 2) °C; (44 ± 0,5) °C

Media tempering temperature: (45 ± 1) °C

The upper incubation temperature limits shall be followed strictly to ensure optimal growth. The lower temperature limits may be exceeded for short periods, e.g. due to opening the door of an incubator, but recovery to the operating temperature should be rapid.

5.2 Incubation times

The following accepted ranges of incubation times are applied, where appropriate for the intended target organism and unless otherwise required in the specific standard.

Incubation times: (21 ± 3) h; (44 ± 4) h; (68 ± 4) h

5.3 Volumes and masses

Measuring equipment has to be appropriate to the required accuracy and precision. The accepted range of any measured value is ±2 % where the stated value is critical to method performance and test results, and ±5 % where the stated value has been shown not to be critical. Examples of critical values having a direct effect on test results are inoculum and diluent volumes, while mass of ingredients used to prepare media are considered not to be as critical.

NOTE Critical tolerances have been set at 2 % to minimize the uncertainty of test results.

6 Diluents and culture media

6.1 General

General requirements for preparation, production, sterilization, storage and performance of culture media are given in ISO 11133.

For preparation of microbiological culture media, unless otherwise stated, add the ingredients to the volume of water, rather than make the ingredients up to a certain volume.

Before use, check the quality of the culture media, diluents, membrane filters and reagents by following the procedures described in ISO 11133 and ISO 7704 or as given in the specific standard.

For information on storage of culture media refer to ISO 11133.

6.2 Quality requirements of ingredients

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

Refer to ISO 11133 and ISO 3696[2] for further information on the quality of ingredients and the quality of water that should be used for media preparation.

6.3 Diluents

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

— Saline solution;

— Peptone diluent;

— Peptone saline solution (MRD);

— Ringer's solution - quarter-strength;

— Phosphate buffer solution.

See Annex D for formulations, preparation, storage and performance testing of these diluents.

7 Sterilization and decontamination

7.1 General apparatus and glassware

Sterilize apparatus and glassware not supplied sterile by one of the following methods:

a) in an oven, operating at (170 ± 10) °C for at least 1 h (excluding pre-heating time);

b) in an autoclave, operating at (121 ± 3) °C for at least 15 min.

Some heat labile items may require sterilization by other means (for example, ultraviolet light or irradiation) but these are not carried out in the routine laboratory.

7.2 Sterilization of consumables

Sterile disposable equipment and materials may be used instead of re-usable items (glassware, Petri dishes, pipettes, bottles, tubes, loops, spreaders etc.) if the specifications are similar.

If membrane filters are not obtained sterile, these are usually sterilized by moist heat according to process b) above, or by following the manufacturer's instructions.

7.3 Decontamination of glassware and materials after use

Materials for decontamination and disposal should be placed in containers, e.g. autoclavable plastic bags. Autoclaving is the preferred method for all decontamination processes (at least 30 min at 121 °C). The autoclave should be loaded in a way that favours heat penetration into the load, (e.g. without over packing).Take care to loosen caps/lids and open bags to prevent dangerous pressurisation of the container which could lead to possible breakage, for example explosion of glass bottles.

Modern autoclaves may not require caps to be loosened, but follow the manufacturer’s instructions strictly to avoid dangerous pressurisation of the containers.

Alternative methods other than autoclaving may be used if allowed by national regulations.

Autoclave all equipment which has been in contact with microbiological cultures (solid or liquid culture media), including re-usable containers prior to being washed.

During examination, decontamination by immersion in freshly prepared disinfectant, prepared at the correct dilution, may be used for small-sized and corrosion resistant equipment (e.g. pipettes).

Pasteur pipettes may be difficult to clean and are usually discarded after a single use.

Most disinfectants have some toxic effects. Wear gloves and eye protection when handling disinfectant and follow the manufacturer’s instructions.

7.4 Waste management

The correct disposal of contaminated materials does not directly affect the quality of sample analysis, but it is a matter of good laboratory management.

It should conform to national environmental or health and safety regulations.

A system for identification and separation of waste materials and their containers should be established for:

— non-contaminated waste (e.g. uncultured water samples) that can be disposed of using general waste streams as allowed by national regulations;

— scalpels, needles, knives, broken glass;

— contaminated materials for autoclaving and recycling;

— contaminated materials for autoclaving and disposal, or for disposal only if the material is to be incinerated.

Incineration of contaminated materials and their contents should be carried out in accordance with national environmental or health and safety regulations.

