



SLOVENSKI STANDARD
oSIST prEN ISO 7704:2021
01-november-2021

[Not translated]

Water quality - Requirements for the performance testing of membrane filters used for direct enumeration of microorganisms by culture methods (ISO/DIS 7704:2021)

Wasserbeschaffenheit - Anforderungen für die Bewertung von Membranfiltern zur direkten Zählung mittels mikrobiologischer Kulturverfahren (ISO/DIS 7704:2021)

Qualité de l'eau - Évaluation des membranes filtrantes utilisées pour des analyses microbiologiques (ISO/DIS 7704:2021)

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Ta slovenski standard je istoveten z: prEN ISO 7704

ICS:

07.100.20 Mikrobiologija vode Microbiology of water

oSIST prEN ISO 7704:2021

en,fr,de

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DRAFT INTERNATIONAL STANDARD

ISO/DIS 7704

ISO/TC 147/SC 4

Secretariat: DIN

Voting begins on:
2021-10-07Voting terminates on:
2021-12-30

Water quality — Requirements for the performance testing of membrane filters used for direct enumeration of microorganisms by culture methods

ICS: 07.100.20

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ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

	Page
Foreword	v
Introduction	vi
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
3.1 General terms and definitions.....	2
3.2 Terminology of performance testing.....	3
3.3 Terminology for test microorganisms.....	4
4 Principle	6
4.1 General.....	6
4.1.1 Batch testing.....	6
4.1.2 Supplementary testing.....	7
4.2 Testing procedures.....	7
4.2.1 Microbiological methods.....	7
4.2.2 Absence of microbial contamination.....	8
5 Apparatus and glassware	8
6 Culture media and diluents	8
7 Preparation of microorganisms for performance testing	8
7.1 General.....	8
7.2 Reference count.....	9
7.2.1 Quantitative productivity testing.....	9
7.2.2 Qualitative selectivity testing.....	10
7.2.3 Qualitative specificity testing.....	10
7.3 Preparation of a standardized test suspension using a working culture.....	10
7.3.1 Preparation of the test suspension (inoculum) for the test.....	10
7.4 Preparation of a test suspension using reference material.....	11
8 Sampling of membrane filters for testing	11
9 Procedure	11
9.1 General.....	11
9.2 Inoculation by spread plate technique.....	12
9.2.1 General.....	12
9.2.2 Inoculation.....	12
9.3 Inoculation by membrane filtration technique.....	12
9.3.1 General.....	12
9.3.2 Inoculation.....	13
9.4 Incubation and counting.....	13
9.5 Test on absence of microbial contamination.....	13
10 Calculation, expression and interpretation of results	13
10.1 General.....	13
10.2 Productivity testing.....	14
10.3 Selectivity testing.....	14
10.4 Specificity testing.....	14
11 Documentation of test results	15
11.1 General.....	15
11.2 Information provided by the manufacturer.....	15
11.3 Traceability.....	15
Annex A (normative) Diagram of the batch testing	16
Annex B (informative) Example of card for recording test results from batch testing	17

ISO/DIS 7704:2021(E)

Annex C (informative) Quantitative supplementary testing of membrane filters	18
Annex D (informative) Qualitative supplementary testing of membrane filters	20
Annex E (informative) Practical example of quantitative batch testing and quantitative supplementary testing by the end user	24
Bibliography	33

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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.

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).

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For an explanation of the voluntary nature of standards, 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 www.iso.org/iso/foreword.html.

This document 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 7704:1985), which has been technically revised.

The main changes compared to the previous edition are as follows:

- the scope has been changed to cover the requirements for the performance testing of membrane filters used for retention and direct enumeration;
- clauses have been added for terms and definitions, microorganisms, sampling and replicates, procedure, inoculation and incubation, counting, calculation and documentation;
- the clauses referencing culture media and diluents, test strain preparation, performance testing and procedure have been revised to align with ISO 8199 and ISO 11133;
- [Annex B](#) has been added to give an example of card for recording test results from batch testing and supplementary testing of membrane filters;
- [Annex C](#) has been added to describe the quantitative additional testing of membrane filters;
- [Annex D](#) has been added to describe the qualitative supplementary testing of membrane filters;
- [Annex E](#) has been added to give a practical example of batch testing and quantitative additional testing by the end user;
- Bibliography has been added;

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

ISO/DIS 7704:2021(E)**Introduction**

This document describes the performance testing of membrane filters used for retention followed by direct enumeration of microorganisms by culture methods.

The main changes listed in the foreword, introduced in this document compared to ISO 7704:1985, are considered as major.

In laboratories carrying out microbiological examinations, the main objectives are to capture, resuscitate, grow, detect and/or enumerate a wide variety of microorganisms. Membrane filters are used in many traditional microbiological culture techniques and are commercially available in various brands and types. Many comparison studies of membrane filters which have been reported in the literature show differences in their ability to recover bacteria from water samples, see References. [18],[19],[23],[25],[27],[28],[29],[30] The complex manufacturing process means that the chemical composition, pore size and pore structure can vary, depending on the brands, and even on the lot of material. Furthermore, the manufacturing process can also release leachables that can potentially interfere with the recovery of microorganisms.

Thus, it is very important to standardize the performance testing of membrane filters as much as possible, not only to provide consistent results, but also to enable the development of standardized procedures for enumerating specific microorganisms.

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Water quality — Requirements for the performance testing of membrane filters used for direct enumeration of microorganisms by culture methods

1 Scope

This document specifies the requirements for the performance testing of membrane filters. This concerns membrane filters which are used for retention followed by direct enumeration of specific microorganisms on solid media or on other devices containing media, like absorbent pads^[16].

It is not applicable for membrane filters used for concentration and elution or for qualitative methods.

These tests are applicable to membrane filters intended for the microbiological analysis of different types of water, like:

- drinking water, bottled water and other types of water with expected low numbers of microorganisms;
- water with expected higher numbers of microorganisms, for example surface water and process water.

These tests are intended to demonstrate the suitability of the whole system - membrane filter together with culture medium including the filtration step - required for the specific tests described in International Standards.

This document applies to:

- manufacturers producing membrane filters,
- microbiological laboratories using membrane filters for their own testing or providing these to other end users.

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 8199:2018, *Water quality — General requirements and guidance for microbiological examinations by culture*

ISO 11133:2014/AMD1:2018, *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:

- ISO Online browsing platform: available at <http://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

ISO/DIS 7704:2021(E)

3.1 General terms and definitions

3.1.1

membrane filter(s)

porous hydrophilic filtration matrix, composed of different polymers with filtration characteristics equivalent to its/their rated nominal pore sizes, typically ranging from 0,1 µm to 1,2 µm, which is intended to be used for retention of microorganisms

Note 1 to entry: The membrane filter assures the effective retention of microorganisms depending on the membrane pore size when a differential (positive or negative) pressure is applied.

Note 2 to entry: The type of membrane filter to be used for a certain microbiological method is described in the corresponding *specific standard* (3.1.7).

3.1.2

culture medium

formulation of substances, in liquid, semi-solid or solid form, which contain natural and/or synthetic constituents intended to support the multiplication (with or without constituents for inhibition of certain microorganisms), identification or preservation of viability of microorganisms

[SOURCE: ISO 11133:2014/AMD1:2018/AMD2:2020, 3.3.1, modified — “constituents for” included and Note 1 to entry has been deleted.]

Note 1 to entry: The culture medium to be used for a certain microbiological method is described in the corresponding *specific standard* (3.1.7), see *specific culture medium* (3.1.3).

3.1.3

specific culture medium

culture medium (3.1.2), usually selective, as designated in a *specific standard* (3.1.7) for use with *membrane filters* (3.1.1)

EXAMPLE Chromogenic Coliform Agar (CCA) used with membrane filters in accordance with ISO 9308-1^[2]; Slanetz and Bartley medium used with membrane filters in accordance with ISO 7899-2^[5].

3.1.4

reference medium

culture medium (3.1.2) for comparative supplementary performance testing

Note 1 to entry: The reference medium is usually a non-selective *culture medium* (3.1.2), which is different to the culture medium under test and demonstrated to be suitable for use in the performance testing.

EXAMPLE Tryptone soya agar (TSA) is in accordance with ISO 11133:2014/AMD1:2018/AMD2:2020.

[SOURCE: ISO 11133:2014/AMD1:2018/AMD2:2020, 3.3.4.13, modified — Note 1 to entry has been implemented.]

3.1.5

batch of membrane filters

lot of membrane filters

homogeneous and fully traceable units of *membrane filter* (3.1.1) referring to a defined amount of bulk, semi-finished product or end product, which is consistent in type and quality and which has been produced within one defined production period, having been assigned the same batch (or lot) number

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3.1.6**batch of culture medium**
lot of culture medium

homogeneous and fully traceable units of a *culture medium* (3.1.2) referring to a defined amount of bulk, semi-finished product or end product, which is consistent in type and quality and which has been produced within one defined production period, having been assigned the same batch (or lot) number

[SOURCE: ISO 11133:2014/AMD1:2018/AMD2:2020, 3.1.2, modified — reference to 3.1.2 has been added.]

Note 1 to entry: If the user does not define the batch or lot of culture medium used for performance testing of membrane filters as the “end product”, it is important to demonstrate that the production process is sufficiently controlled to produce culture media of consistent quality (for example by monitoring culture media performance testing results).

3.1.7**specific standard**

International Standard or guidance document describing the microbiological analysis of different types of water for the detection or enumeration of a specific microorganism (or group of microorganisms)

[SOURCE: ISO 6887-1:2017, 3.10, modified]

3.2 Terminology of performance testing**3.2.1****batch testing**

test of units of *membrane filters* (3.1.1) from a *batch* or a *lot* (3.1.5) for its/their intended use with the *specific culture medium* (3.1.3) and determination of *reference count* (3.3.6)

3.2.2**supplementary quantitative testing**

test of units of *membrane filters* (3.1.1) from a *batch* or a *lot* (3.1.5) for its/their intended use with the *specific culture medium* (3.1.3); determination of *reference count* (3.3.6); test of the membrane filters on a non-selective culture medium and test of the culture medium without membrane filter

Note 1 to entry: A detailed description of the supplementary quantitative testing is given in [Annex C](#).

Note 2 to entry: Supplementary quantitative testing is used when a new type of a *membrane filter(s)* (3.1.1), or a new manufacturer is tested initially, or when a problem in the day-to-day use or batch testing of membrane filters is noticed.

3.2.3**performance of membrane filter**

response of a *membrane filter* (3.1.1) challenged by a *test organism* (3.3.1) under defined conditions

Note 1 to entry: The defined conditions are described in the *specific standard* (3.1.7) for the intended use of the *membrane filter* (3.1.1), like: *test organism* (3.3.1), *specific culture medium* (3.1.3), *reference medium* (3.1.4), *productivity* (3.2.7), *selectivity* (3.2.6), incubation time and temperature.

3.2.4**performance of culture medium**

response of a *culture medium* (3.1.2) challenged by a *test organism* (3.3.1) under defined conditions

[SOURCE: ISO 11133:2014/AMD1:2018/AMD2:2020, 3.2.1]

3.2.5**productivity**

level of recovery of a *target microorganism* (3.2.5) on the *culture medium* (3.1.2) with the *membrane filter* (3.1.1) under defined conditions

[SOURCE: ISO 11133:2014/AMD1:2018/AMD2:2020, 3.2.4, modified — adapted to the performance testing of membrane filters.]

ISO/DIS 7704:2021(E)

3.2.6

selectivity

degree of inhibition of a *non-target microorganism* (3.2.6) on a selective *culture medium* (3.1.2) with the *membrane filter* (3.1.1) under defined conditions

[SOURCE: ISO 11133:2014/AMD1:2018/AMD2:2020, 3.2.5, modified — adapted to the performance testing of membrane filters.]

3.2.7

electivity

specificity

demonstration, under defined conditions, that a *non-target microorganism* (3.2.6), if able to grow on the medium, does not show the same visual characteristics as the *target microorganisms* (3.2.5) on the *culture medium* (3.1.2) with *membrane filter* (3.1.1) under defined conditions

[SOURCE: ISO 11133:2014/AMD1:2018/AMD2:2020, 3.2.6, modified — adapted to the performance testing of membrane filters.]

3.3 Terminology for test microorganisms

3.3.1

target microorganism

microorganism or group of microorganisms to be detected or enumerated which should be expected to grow under defined conditions [SOURCE: ISO 11133:2014/AMD1:2018/AMD2:2020, 3.2.2], modified

3.3.2

non-target microorganism

microorganism that is suppressed by the medium and/or conditions of incubation or does not show expected characteristics of the target microorganism

[SOURCE: ISO 11133:2014/AMD1:2018/AMD2:2020, 3.2.3]

3.3.3

test microorganism

control strain

microorganism generally used for performance testing of *membrane filters* (3.1.1) and/or *culture media* (3.1.2)

Note 1 to entry: Test microorganisms / control strains are further defined in accordance with their source (see 3.3.2 to 3.3.8).

[SOURCE: ISO 11133:2014/AMD1:2018/AMD2:2020, 3.4.1, modified — adapted to the performance testing of membrane filters.]

3.3.4

reference strain

microorganism obtained directly from a reference culture collection and defined to at least the species level

Note 1 to entry: A reference culture collection is a culture collection which is a member of the World Federation of Culture Collections (WFCC) or the European Culture Collections' Organisation (ECCO).

Note 2 to entry: A reference strain is catalogued and described according to its characteristics and preferably originating from water as applicable.

[SOURCE: ISO 11133:2014/AMD1:2018/AMD2:2020, 3.4.2, modified — Note 1 and 2 to entry have been implemented using modified text from shortened definition.]

3.3.5**reference stock**

set of separate identical cultures obtained by a single subculture from the *reference strain* (3.3.4) either in the laboratory or from a supplier

[SOURCE: ISO 11133:2014/AMD1:2018/AMD2:2020, 3.4.3]

3.3.6**stock culture**

primary subculture from a *reference stock* (3.3.5)

[SOURCE: ISO 11133:2014/AMD1:2018/AMD2:2020, 3.4.4]

3.3.7**working culture**

subculture from a *reference stock* (3.3.5) or *stock culture* (3.3.6) or a *reference material* (3.3.9) or a *certified reference material* (3.3.10)

Note 1 to entry: Multi-strain *reference material* (3.3.9) and multi-strain *certified reference material* (3.3.10) can also be used for the preparation of working cultures.

[SOURCE: ISO 11133:2014/AMD1:2018/AMD2:2020, 3.4.4, modified — Note 1 to entry has been added.]

3.3.8**reference count**

total count of colonies on a mostly non-selective *culture medium* (3.1.2) obtained without usage of a *membrane filter* (3.1.1)

Note 1 to entry: For this the term “inoculum level” is often used.

3.3.9**reference material
RM**

microbiological material containing a quantity of revivable microorganisms, sufficiently homogenous and stable with respect to the quantity of revivable microorganisms, which has been established to be fit for its intended use in a measurement process

Note 1 to entry: See ISO Guide 30^[12].

[SOURCE: ISO 11133:2014/AMD1:2018/AMD2:2020, 3.4.6, modified — Note 1 to entry: reference has been updated.]

Note 2 to entry: For in-house prepared quality control reference materials (QRMs), often so-called “in-house reference materials” or “internal RM”, see ISO Guide 80^[15] and References [20],[24].

3.3.10**certified reference material
CRM**

microbiological *reference material* (3.3.9) characterized by a metrologically valid procedure for the quantity of revivable microorganisms

Note 1 to entry: See ISO Guide 30^[13].

Note 2 to entry: Metrologically valid procedures for the production and certification of RMs are given in, among others, ISO 17034^[12] and ISO Guide 35^[14].

Note 3 to entry: A microbiological CRM is accompanied by a certificate that provides the value of the specified quantity of revivable microorganisms, its associated uncertainty and a statement of metrological traceability. A CRM is certified only for the method/methods and media that were included in the certification process, otherwise its function is like an (ordinary) *reference material* (3.3.9).