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

Qualité de l'eau — Exigences relatives aux essais de performance des membranes filtrantes utilisées pour le dénombrement direct des micro-organismes par des méthodes de culture

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Foreword

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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,* in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 230, *Water analysis,* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This second edition cancels and replaces the first edition (ISO 7704:1985), which has been technically revised.

The main changes 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 to culture media and diluents, test strain preparation, performance testing and procedure have been revised to align with ISO 8199 and ISO 11133;

— Annex A has been added with a diagram of the batch testing;

— Annex B has been added to give an example of a card to record the 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 including a diagram of the procedure;

— 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 including a diagram of the procedure;

— the 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.

Introduction

In laboratories carrying out microbiological examinations, the main objectives are to either capture, resuscitate, grow, detect or enumerate, or all, 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 [22], [23], [28], [30], [31], [32], [33] and [34]. 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.

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 used for the retention followed by direct enumeration of microorganisms by culture methods.

This document is applicable to 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[19].

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

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

— 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 the culture medium including the filtration step) required for the specific tests described in References [3], [6], [8], [10], [12] and [13].

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

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 terminology databases for use in standardization at the following addresses:

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

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

3.1 General terminology

3.1.1
membrane filter
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 the retention of microorganisms

Note 1 to entry: The membrane filter ensures 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 either 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

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).

[SOURCE: ISO 11133:2014, 3.3.1, modified — "constituents for" has been included and Note 1 to entry has been replaced.]

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 1 Chromogenic coliform agar (CCA) used with membrane filters in accordance with ISO 9308-1[8].

EXAMPLE 2 Slanetz and Bartley medium used with membrane filters in accordance with ISO 7899-2[6].

3.1.4
reference medium
culture medium (3.1.2), usually non-selective, for determination of the *reference count* (3.3.8) and *supplementary quantitative testing* (3.2.2)

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 has been demonstrated to be suitable for use in the performance testing.

EXAMPLE Tryptone soya agar (TSA) is in accordance with ISO 11133.

[SOURCE: ISO 11133:2014, 3.3.4.13, modified — Note 1 to entry has been added and "is in accordance with ISO 11133" has been added in the example.]

3.1.5
batch of membrane filter
lot of membrane filter
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

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

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 (e.g. by monitoring culture media performance testing results).

[SOURCE: ISO 11133:2014, 3.1.2, modified — "medium" has been changed to "culture medium" and Note 1 to entry has been added.]

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)

3.2 Terminology of performance testing

3.2.1
batch testing
test of units of *membrane filters* (3.1.1) from a *batch of membrane filter* (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 of membrane filter* (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: Annex C gives detailed description of the supplementary quantitative testing.

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* (3.2.1) of membrane filters is noticed.

3.2.3
performance of the membrane filter
response of a *membrane filter* (3.1.1) challenged by a *test organism* (3.3.3) 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* (3.3.3), *specific culture medium* (3.1.3), *reference medium* (3.1.4), *productivity* (3.2.5), *selectivity* (3.2.6), *specificity* (3.3.7), incubation time and temperature.

3.2.4
performance of the culture medium
response of a *culture medium* (3.1.2) to challenge by *test organisms* (3.3.3) under defined conditions

[SOURCE: ISO 11133:2014, 3.2.1]

3.2.5
productivity
level of recovery of a *target microorganism* (3.3.1) from the *culture medium* (3.1.2) with the *membrane filter* (3.1.1) under defined conditions

[SOURCE: ISO 11133:2014, 3.2.4, modified — "with the membrane filter" has been added to the term and the definition.]

3.2.6
selectivity
degree of inhibition of a *non-target microorganism* (3.3.2) on or in a selective *culture medium* (3.1.2) with the *membrane filter* (3.1.1) under defined conditions

[SOURCE: ISO 11133:2014, 3.2.5, modified — "with the membrane filter" has been added to the term and the definition.]

3.2.7
specificity
demonstration, under defined conditions, that *non-target microorganisms* (3.3.2), if able to grow on the medium, do not show the same visual characteristics as the *target microorganisms* (3.3.1) on the *culture medium* (3.1.2) with *membrane filter* (3.1.1) under defined conditions

3.3 Terminology for test microorganisms

3.3.1
target microorganism
microorganism or group of microorganisms to be detected or enumerated which can be expected to grow under defined conditions

[SOURCE: ISO 11133:2014, 3.2.2, modified — "which can be expected to grow under defined conditions" has been added to the definition.]

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, 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 according to their source (see 3.3.4 to 3.3.10).

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' Organization (ECCO).

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

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, 3.4.3]

3.3.6
stock culture
primary subculture from a *reference stock* (3.3.5)

[SOURCE: ISO 11133:2014, 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, 3.4.4, modified — "certified or not" has been replaced with " or a *certified reference material*" Note 1 to entry has been added.]

3.3.8
reference count
inoculum level
total count of colonies on a *reference medium* (3.1.4) obtained without usage of a *membrane filter* (3.1.1)

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[15].

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[18] and References [23], [25], [27] and [28].

[SOURCE: ISO 11133:2014, 3.4.6, modified — the reference in Note 1 to entry has been updated and Note 2 to entry has been added.]

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[15].

Note 2 to entry: Metrologically valid procedures for the production and certification of RMs are given in, among others, ISO 17034,[14] ISO Guide 31[16] and ISO Guide 35[17].

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).

4 Principle

4.1 General

4.1.1 Introduction

Membrane filters are used for retention followed by direct enumeration of microorganisms in water samples. Many materials, pore sizes and brands are commercially available and designed for specific

